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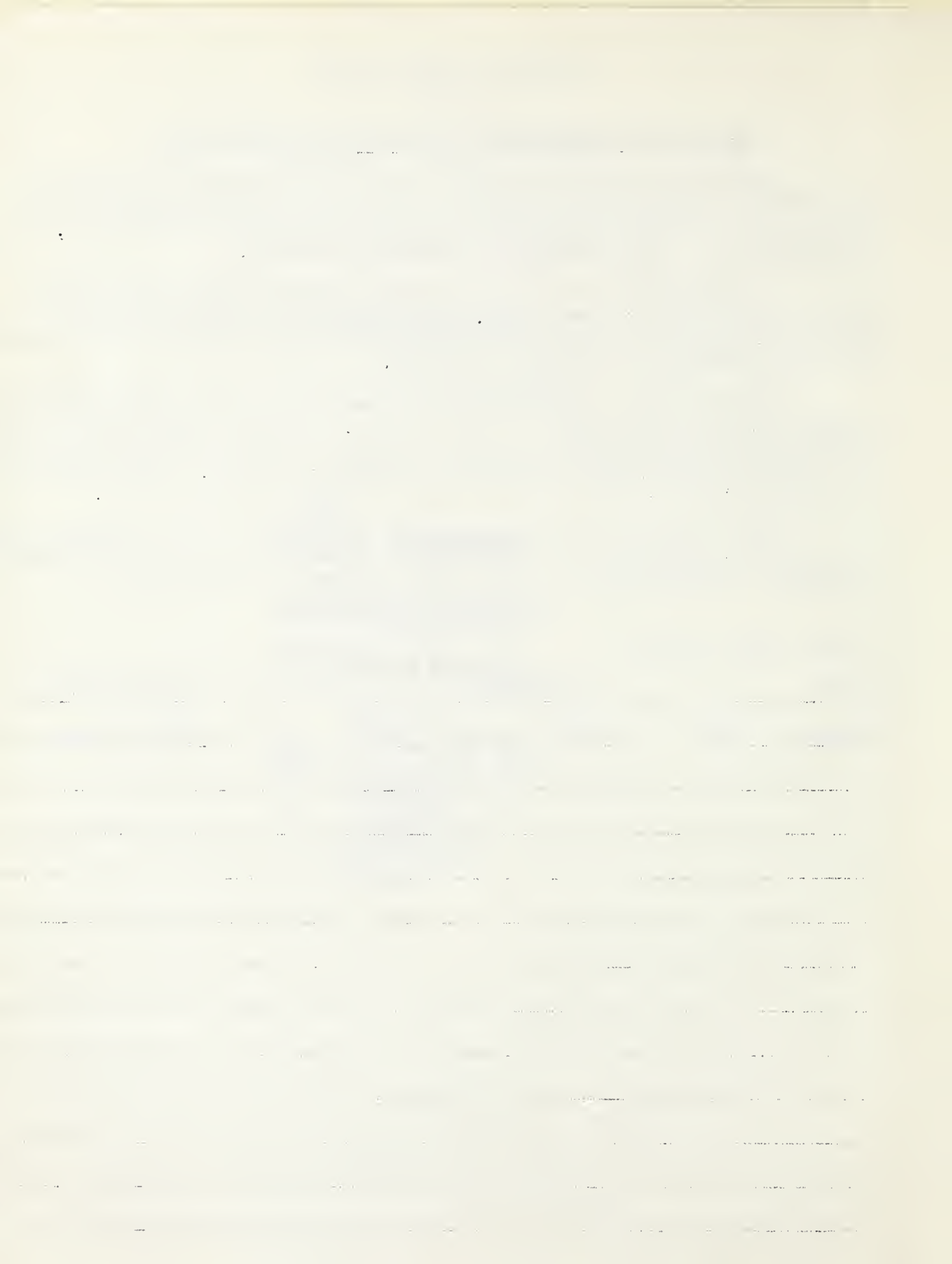
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THE UNIVERSITY OF ALBERTA

THE INFLUENCE OF RESERPINE ON PERIPHERAL  
VASCULAR RESPONSES TO HYPERCAPNIA

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE  
OF MASTER OF SCIENCE

DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY

by

ROBERT JOHN SHIVAK

EDMONTON, ALBERTA

1961



UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "The Influence of Reserpine on Peripheral Vascular Responses to Hypercapnia" submitted by Robert John Shivak in partial fulfilment of the requirements for the degree of Master of Science.

Date

July 18/61



## ABSTRACT

The peripheral vascular response to noradrenaline and pitressin is depressed during hypercapnia, which also appears to cause a release of noradrenaline from tissue stores. The hypothesis that the depression of the response to noradrenaline is due to partial saturation of the adrenergic receptors was tested in two series of perfusion experiments in reserpinized and unreserpinized dogs and cats. Since reserpine pretreatment of animals has been shown to deplete the vascular tissue stores of noradrenaline it was postulated that if the hypercapnic depression of noradrenaline was due to saturation of the adrenergic receptors it would not be observed in reserpinized animals.

The peripheral vascular responses to noradrenaline, tyramine and pitressin were tested in both reserpinized and unreserpinized animals before and during hypercapnia. Tyramine was used as a test of depletion of noradrenaline stores, while pitressin was used as an example of a drug having a direct action on vascular musculature which is uninfluenced by adrenergic receptor blockade.

The hypercapnic depression of both noradrenaline and pitressin was observed in both reserpinized and unreserpinized animals. It was therefore concluded that the depression was effected at some point in the contractile process beyond the postulated adrenergic receptor site.



To my wife  
Joanne Patricia



## ACKNOWLEDGEMENTS

To Dr. C. W. Nash, my supervisor, I extend my sincere appreciation for the help and advice given to me throughout the course of my work.

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## TABLE OF CONTENTS

CHAPTER	PAGE
I. INTRODUCTION .....	1
II. LITERATURE REVIEW .....	4
1. Control of Acid Base Balance .....	4
2. $p^H$ and Smooth Muscle Activity .....	9
3. The Effect of Carbon Dioxide and $p^H$ Changes on the Cardiovascular System .....	12
4. Depression of Catecholamine Activity During Acidosis .....	19
5. Pharmacological Actions of Reserpine and its Influence on Noradrenaline and Tyramine .....	21
6. Action of Pitressin on Smooth Muscle .....	25
III. EXPERIMENTAL METHODS .....	26
IV. RESULTS .....	29
V. DISCUSSION .....	45
VI. SUMMARY AND CONCLUSIONS .....	56
VII. BIBLIOGRAPHY .....	58
VIII. APPENDICES .....	67



# LIST OF TABLES

TABLE		PAGE
I.	Pressure, Flow and Resistance in Hind Limbs of Untreated and Reserpine Pretreated Dogs Before and During Hypercapnia .....	30
II.	A Comparison of the Percentage Elevation of Peripheral Resistance Caused by the Test Drugs in Untreated and Reserpine Pretreated Dogs .....	32
III.	The Effect of Adrenergic Blockade on the Peripheral Vascular Responses of Dogs to Hypercapnia .....	35
IV.	The Effect of Reserpine on the Peripheral Vascular Responses of Dogs to Hypercapnia .....	37
V.	Theoretical Maximum Responses to I.A. Doses of Noradrenaline Before and During Carbon Dioxide in Untreated Spinal Cats .....	40
VI.	Theoretical Maximum Responses to I.A. Doses of Noradrenaline Before and During Carbon Dioxide in Reserpine Pretreated Spinal Cats .....	42
VII.	Pressor Responses to 40 $\mu$ g Tyramine I.A. in Untreated Cats Before and During CO <sub>2</sub> .....	43
VIII.	Pressor Responses to 40 $\mu$ g Tyramine I.A. in Reserpine Pretreated Cats Before and During CO <sub>2</sub> .....	43
IX.	Pressor Responses to 0.1 Unit Pitressin I.A. in Untreated Cats Before and During CO <sub>2</sub> .....	44
X.	Pressor Responses to 0.1 Unit Pitressin I.A. in Reserpine Pretreated Cats Before and During CO <sub>2</sub> ....	44



## LIST OF FIGURES

FIGURE		PAGE
1.	Peripheral Vascular Responses of Reserpine Treated and Untreated Dogs Before and During Hypercapnia .....	33
2.	Noradrenaline Dose-Response Relationships in the Perfused Hind Limbs of Untreated Cats .....	39
3.	Noradrenaline Dose-Response Relationships in the Perfused Hind Limbs of Reserpine Pretreated Cats .....	41



## INTRODUCTION

This investigation is an attempt to determine the mechanism of the depression of the vascular response to noradrenaline which occurs during hypercapnia.

A number of workers (1 to 11) have indicated that variations in the pH of blood or of organ bath fluid can cause alterations in responses of smooth muscle preparations to adrenaline and noradrenaline. Some of these variations in blood pH were brought about by alterations in the ventilation of animals so that the altered responses to noradrenaline may have been due to changes in the carbon dioxide content as well as of the pH of the blood and tissues.

Hypercapnia causes an increase in blood pressure and a decrease in the pressor response to noradrenaline. Miller (12) has shown that respiratory acidosis caused by diffusion respiration is accompanied by an increase in circulating catecholamines. The blood pressure first rises but then falls to the original level although the plasma catecholamine concentration is still increasing. It is known that infusions of noradrenaline at first cause a rise in pressure but the pressure will fall to the original level while the infusion is still being made.(13,14) If a test dose of noradrenaline is given following such an infusion the response is lower than it was before. J. H. Burn (13) has postulated this reduced sensitivity is a result of saturation of adrenergic receptors, "leaving few receptors free on which noradrenaline present in the blood stream can act."



If the Burn explanation is correct, the intrinsic release of vascular noradrenaline by hypercapnia may cause a partial saturation of adrenergic receptors and thus cause the lowered sensitivity to injected noradrenaline observed during hypercapnia.

Treatment with reserpine has been shown to cause the loss of a noradrenaline-like substance from vascular tissue leaving the tissues more sensitive to injected noradrenaline but less sensitive to tyramine (15,16). On the contrary, five minutes after an intravenous dose of reserpine, rats and rabbits have been shown to be less sensitive to noradrenaline but more responsive to tyramine (14) which Burn and Rand postulate acts by release of noradrenaline from vascular tissue stores. For these reasons it was postulated that if the depression of the noradrenaline response caused by hypercapnia was due to noradrenaline release and partial saturation of the adrenergic receptors, pretreatment with reserpine may abolish this hypercapnic depression. The tissue stores of noradrenaline would then be depleted and most of the adrenergic receptors free, thus reducing the probability of a saturation of receptor sites during the hypercapnia.

It was also postulated that if hypercapnia caused a release of noradrenaline from vascular tissue stores it may, like reserpine, potentiate the action of tyramine. In addition, it was assumed that a decrease in response to injected tyramine would serve as an indication of the magnitude of depletion of noradrenaline stores by pretreatment with reserpine.

Finally, it was postulated that if the hypercapnic depression of



the response to noradrenaline was unrelated to the receptor mechanism but functioned at some point between the receptor and the effector mechanism of the contractile process, the response to pitressin would be depressed by hypercapnia but unaffected by pretreatment with reserpine.

To test these hypotheses two series of experiments were performed.

I. The blood pressure and flow in the hind limbs of pentobarbital anaesthetized dogs were measured and the percentage changes in peripheral resistance induced by small intra-arterial doses of noradrenaline, tyramine and pitressin were calculated. These changes were measured in reserpinized and non-reserpinized animals before and during hypercapnia.

II. The hind limbs of spinal cats were perfused with their own blood, flow being kept constant by use of a Sigmamotor pump. A series of test doses of noradrenaline was given intra-arterially and the pressor responses measured as an indication of resistance changes. Dose-response curves were plotted and the changes induced by the hypercapnia in reserpine treated and untreated animals were compared.



## LITERATURE REVIEW

### 1. Control of Acid Base Balance

Three different but interrelated mechanisms serve to minimize variations in the hydrogen ion concentration in mammals. These are:

1. Buffer systems of the blood and tissues.
2. Respiratory regulation.
3. Renal regulation.

#### 1. The buffer systems.

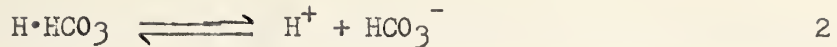
Buffer systems are characterized by their ability to resist changes in hydrogen ion concentration. The most active blood buffer is the hemoglobin:oxyhemoglobin system, since for each millimole of hemoglobin reduced, 0.7 millimole of hydrogen ions is taken up. Minor roles are also played by the monopotassium phosphate:dipotassium phosphate system and the carbonic acid:bicarbonate system.

The most important regulator of blood pH is the bicarbonate: carbonic acid system. This system is a poor buffer due to the 20:1 ratio between its constituents but plays the major role in pH regulation because carbon dioxide concentration, and thus the carbonic acid concentration is altered by the respiratory mechanism and the bicarbonate ion concentration is controlled by the kidney. Carbon dioxide is formed in the body as an end-product of catabolism. It combines reversibly with water to produce carbonic acid,





which dissociates into hydrogen and bicarbonate ions.



For weak electrolytes, the hydrogen ion concentration is calculated from the Law of Mass Action expressed by the equation

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} \quad 3$$

in which  $[\text{H}^+]$  represents the cation or hydrogen ion concentration,  $[\text{A}^-]$  represents the anion concentration,  $[\text{HA}]$  represents the concentration of undissociated acid and  $K_a$  is the dissociation or ionization constant of the acid. The ratio of the products of the concentrations of the ionized components to that of the unionized is equal to a constant  $K_a$  which expresses the acid strength in terms of its tendency to dissociate into positive hydrogen ions and negative anions. Using carbonic acid as the specific acid, equation 3 may be rearranged as follows,

$$K_a [\text{H}\cdot\text{HCO}_3] = [\text{H}^+][\text{HCO}_3^-] \quad 4$$

Carbonic acid will form bicarbonate salts,  $\text{B}\cdot\text{HCO}_3$ , by reacting with the various basic cations of the blood buffers. Dissociation of the weakly ionized carbonic acid into hydrogen and bicarbonate ions is depressed by the presence of excess bicarbonate ions, resulting from the dissociation of the relatively highly ionized bicarbonate salts.



Thus, most of the bicarbonate ions in blood are derived from dissociation of the bicarbonate salts. The preponderance of bicarbonate ions causes the equilibrium in equation 5 to shift to the left, resulting



in a greater concentration of undissociated carbonic acid and a  
decreased concentration of hydrogen ions. Since the almost completely  
dissociated bicarbonate salts are the major source of bicarbonate ions,  
equation 4 may be changed to

$$K_a [H \cdot HCO_3] = [H^+] [B \cdot HCO_3] \quad 7$$

Rearranging equation 7 gives Henderson's equation

$$[H^+] = K_a \times \frac{[H \cdot HCO_3]}{[B \cdot HCO_3]} \quad 8$$

Sorensen introduced the concept of pH, which may be defined as  
the negative logarithm of the hydrogen ion concentration. Hasselbalch  
transformed Henderson's equation into its negative logarithmic form

$$-\log[H^+] = -\log \left( K_a \times \frac{[H \cdot HCO_3]}{[B \cdot HCO_3]} \right) \quad 9$$

Substituting pH and pKa for their negative logarithmic terms results in

$$pH = pK_a - \log \frac{[H \cdot HCO_3]}{[B \cdot HCO_3]} \quad 10$$

Replacing  $-\log \frac{[H \cdot HCO_3]}{[B \cdot HCO_3]}$  by its equivalent,

$$+ \log \frac{[B \cdot HCO_3]}{[H \cdot HCO_3]}, \text{ results in the Henderson-Hasselbalch}$$

equation,

$$pH = pK_a + \log \frac{[B \cdot HCO_3]}{[H \cdot HCO_3]} \quad 11$$

Normal blood bicarbonate is 27 m Eq. per liter and the carbonic  
acid concentration is 1.35 m Eq. per liter. The bicarbonate:carbonic  
acid ratio, therefore, is 20:1. If the ratio is preserved at 20:1,  
the pH will be normal no matter what changes occur in concentration.  
A decreased ratio results in a decreased pH or acidosis. (17)



## 2. The respiratory regulation.

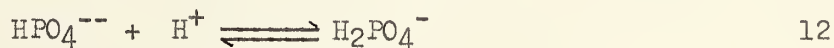
From the Henderson-Hasselbalch equation it is evident that a fall in  $pH$  is compensated if alveolar ventilation is increased and  $[CO_2] + [H_2CO_3]$  consequently decreases. Correspondingly a rise in  $pH$  can be limited by a simultaneous increase of  $[CO_2] + [H_2CO_3]$ , i.e. by a reduced excretion of carbon dioxide. Thus hyperventilation causes a rise in blood  $pH$  while hypoventilation or breathing carbon dioxide will cause a fall in blood  $pH$ .

## 3. Renal regulation.

Through the renal mechanism the body can excrete non-volatile acids and bases. Most of the filtered  $HCO_3^-$  is reabsorbed in the proximal tubules together with equivalent amounts of  $Na^+$ .  $H^+$  is excreted in the distal and collecting tubules by exchange with  $Na^+$ .

The production of  $H_2CO_3$  is accelerated by an increasing carbon dioxide tension and by carbonic anhydrase, while it is inhibited by a reduced  $pCO_2$ .

The  $H^+$  which is exchanged for  $Na^+$  is buffered in the urine in the following manner:



Finally the hydrogen ions may be excreted as the acid  $NH_4^+$ , since  $NH_3$  is produced in the tubular cells by deamination of glutamic acid, and this base binds  $H^+$  as  $NH_4^+$  in the tubular lumen. (18,19)

Conditions in which the acid-base balance is altered may be classified as "metabolic" type or "respiratory" type. In both types, both carbon dioxide and bicarbonate ions may be altered but in the



metabolic type of alteration the primary factor is a change in bicarbonate concentration by other than respiratory means.

In the respiratory type of alteration of the acid-base balance there is a change in the carbonic acid concentration due to ventilatory retention or "blowing off" of carbon dioxide. Respiratory acidosis is due to an excess of carbon dioxide; the bicarbonate:carbonic acid ratio is decreased, resulting in a decreased  $pH$  or acidosis. In respiratory alkalosis, the bicarbonate:carbonic acid ratio is increased due to a deficit of carbon dioxide, thus the  $pH$  is increased or alkalosis is present. (17)



## 2. pH and Smooth Muscle Activity

Many investigations on the effects of acid or alkali on plain muscle from various sources have been reported. (20-25) In most of the published work, the effects of change of reaction on the tonus, or on the spontaneous rhythmic contractions, of some particular preparation have been studied. However, there is a lack of unanimity as regards the effect. Although the musculature of the blood vessels is generally considered to be relaxed by increase, and constricted by decrease of hydrogen-ion concentration, McDowall (21) demonstrated that acids may either constrict or dilate blood vessels depending on the conditions that prevail at the time. An explanation for this discrepancy may be that in the perfused preparation the hydrogen-ion concentration and oxygen supply of the perfusion fluid were abnormal, with the consequence that the vessels started in a state of maximal dilatation, which was not further influenced by small amounts of acid, but was replaced by constriction when an acid reaction, far beyond any obtainable in vivo, was reached.

The tissues employed by Evans and Underhill (22) were the uterus and intestine of the cat, guinea pig and rabbit, the sphincter of the iris of the cat, and the retractor penis of the dog.

The method employed by these investigators was to suspend the plain muscle in a saline bath at 38° C. Oxygen was bubbled at a constant rate through the bath. The reaction of the bath was altered by the addition of acid or alkali.

Evans and Underhill found that the effect of increase of hydrogen-ion concentration was to cause relaxation of the tone of plain muscle.



Rhythmic contractions, such as those of the small intestine, were slowed and depressed in amplitude on the acid side of neutrality, and quickened and depressed when the reaction was made alkaline. The authors suggest that these effects are independent of action on local nervous mechanisms, since it was found that the effect of change of reaction in the presence of 0.01% atropine was essentially the same, as in the absence of it.

Furchgott and Wales (23) reported on the contractile activity of longitudinal smooth muscle of rabbit small intestine in media varying in pH from 5.35 to 7.4. Constant temperature baths at 37.5°C were used with continuous aeration with 95% oxygen and 5% carbon dioxide. The smooth muscle was suspended in a Krebs-Henseleit stock solution and the pH of the media was varied by changing the bicarbonate concentration.

The authors found that spontaneous contractile activity of the muscle occurred at all pH levels studied. However, at the lowest levels, pH 5.35 to 5.75, the amplitude and frequency were markedly lower than at pH 7.4, and the contraction pattern was very irregular. The authors conclude that the reduced activity at low pH levels does not result from injury to the contractile system of the muscle since acetylcholine gave increases in tone equal in magnitude to increases given by comparable segments at pH 7.4. It was found, however, that prolonged exposure of over sixty minutes to media of low pH gave irregular contraction patterns even when the pH of the media was raised to 7.4.

An interesting point demonstrated by these investigators is that glucose is not capable of yielding energy for contraction when added to intestinal segments in media at pH levels below the physiological range. It is suggested that the decrease in effectiveness of the added



glucose results chiefly from a decrease in activity of hexokinase necessary for the transferring of glucose from the medium into smooth muscle cells.

It should be noted that in the works cited up to this point, the effect of  $pH$  on the activity of smooth muscle was studied by the addition of the appropriate acid or alkali to the medium. The question arises whether the effect is the same whether the increase of acidity is caused by carbon dioxide or by an acid, no carbon dioxide being present.

Fraser (24) working with isolated rabbit intestine found that in the presence of carbon dioxide, the contractions were nearly always suppressed at  $pH$  6.4, on the other hand, using  $HCl$ , it was necessary to lower the  $pH$  to 5.4 to achieve the same amount of depression. Fraser found that the strips generally recovered completely when normal  $pH$  was restored after exposure to carbon dioxide. The recovery after  $HCl$  was much less complete.

The work of Sollmann et al (25) agrees in principle with that of Fraser. As in the previous work, small intestines of rabbits were used. The authors found that carbon dioxide causes depression at about  $pH$  6.4, and arrest generally between 6.2 and 6.0. The depression caused by  $HCl$  was found to be quantitatively the same as that caused by carbon dioxide.

In summary, when the medium surrounding a smooth muscle preparation is made acidic, either by the addition of an acid or by bubbling carbon dioxide through it, the effect is similar. Both acid and carbon dioxide depress and slow the contractile activity of the muscle.



### 3. The Effect of Carbon Dioxide and pH Changes on the Cardiovascular System

In determining the effect of acidity on the heart, all of the earlier work was performed on the isolated preparation. Gaskell (26) observed that when a 1:10,000 solution of lactic acid in normal saline was perfused through a frog's heart, it quickly brought the ventricle to a standstill in the position of complete relaxation. In 1913, Mines (27) reported a slowing of A-V conduction when a frog's heart was perfused with normal saline at  $[H] 10^{-7}$ , while perfusion with normal saline at  $[H] 10^{-5}$  caused partial block, complete block and finally cardiac arrest after ten minutes of perfusion. In 1922 Andrus and Carter (28) observed that a perfused terrapin's heart would stop beating at pH 7.1. Daly and Clark (29) reported that in the frog's heart the effect of "feebly acid" Ringer's solution was to reduce the force of beat and to impair conduction from auricle to ventricle. These results agree with those of Mines'. (27).

From a survey of the available literature it would appear that Andrus and Carter (30) were one of the first ones to study the effect of pH on the mammalian heart. Andrus and Carter perfused the dog's heart through the brachio-cephalic artery under constant temperature and pressure. They reported that the heart will function virtually unimpaired between pH 7.0 and pH 7.8. When the pH was lowered below pH 7.0 the rate was markedly slowed and A-V and intraventricular conduction were prolonged. When the perfusion was maintained at this level for several minutes, partial heart block, complete heart block and cessation of



heart beat in diastole supervened in that order.

In further work on perfused dog's heart, Drury and Andrus (31) demonstrated that the transmission rate of excitation in auricular muscle is decreased by perfusates below pH 7.0.

In 1946, Gertler and Hoff (32) reported the results of investigations conducted on dogs made acidotic by means of intravenous infusion of either lactic or hydrochloric acid. They found the lethal level of pH in the dog averaged 6.2.

In the works cited above pH was altered by either lactic or hydrochloric acid. It is of interest to note that the effect of carbon dioxide on the heart was studied as early as 1910 by Jerusalem and Starling. (33) These authors reported a slowing of the isolated dog heart by excessive carbon dioxide. Whitehorn and Bean (34) showed that the administration of a gas mixture containing 11.5% carbon dioxide in oxygen at atmospheric pressure to dogs resulted in an initial brief acceleration followed by a pronounced depression in both the heart rate and the A-V conduction. With the isolated Guinea Pig heart McElroy et al (35) found that when the carbon dioxide tension was increased and the perfusate became more acid, there was a decrease in contraction amplitude, decrease in rate and an increase in coronary flow.

Miller et al (36) induced respiratory acidosis of sufficient magnitude to depress the arterial blood pH of dogs to values ranging from 7.0 to 6.7. The only consistent changes they were able to distinguish in the electrocardiograms made on these acidotic dogs was a marked bradycardia and an increased T-wave amplitude.

It has been demonstrated that permanent cardiac arrest rarely



occurs during stimulation of the vagus nerve in an animal under normal conditions. (37) However, Sloan (38) has shown that in the presence of hypercapnia in dogs induced by breathing 14.3 per cent to 33.2 per cent carbon dioxide, vagal stimulation is accompanied by marked hypotension and occasionally death.

Miller et al (39) noted that in dogs exposed to increasing carbon dioxide concentrations there was an initial drop in blood pressure followed by a slow progressive rise plus the expected hyperventilation. When a level of sixty per cent carbon dioxide was reached the respiration ceased. If respiration was maintained artificially, the concentration of carbon dioxide could be raised to seventy per cent before the blood pressure fell and before the animal demonstrated evidence of electrocardiographic changes. If these animals were suddenly returned to breathing room air they regularly developed ventricular fibrillation and died provided the  $pH$  rose rapidly. More recently, Heath and Brown (40) \* demonstrated a marked reduction in cardiac output of dogs following two hours of hypercapnia.

The decrease in force of contraction with a decrease in  $pH$  of the perfusing fluid may be related to the effect of  $pH$  on potassium movement into and out of muscle cells. In isolated frog skeletal muscle, Fenn and Cobb (41) found a movement of potassium toward the <sup>more</sup>poorly buffered side of the cell membrane when  $pH$  was decreased. When the muscle was bathed in Ringer's solution and the  $pH$  decreased, potassium came out of the muscle. When the bathing fluid was blood a reverse movement of potassium was observed on lowering the  $pH$ . On the other hand, plasma potassium concentrations in cats have been found to increase during hypercapnia (42).

\*A marked increase in cardiac output was observed in the early stages of hypercapnia.



Mackay (43) found that adrenalectomy did not prevent the rise in potassium during hypercapnia but that evisceration abolished it.

The fact that acidosis reduces tissue glucose utilization may be a cause of the depression of cardiac activity during acidosis. This effect may be due to the inhibition of processes of phosphorylation that are involved in the uptake of glucose and potassium and phosphorous by the cells of the body (44,45).

The effect of carbon dioxide on the coronary circulation has been the subject of several papers. Markwalder and Starling (46) using a dog heart-lung preparation observed that increased tension of carbon dioxide in the blood caused dilatation of the coronary vessels associated with a dilatation of the heart. Other publications (47, 48, 49) reported work which agrees with that of Markwalder and Starling, but some authors (50, 51) were unable to find any significant alteration of coronary flow with concentrations of carbon dioxide up to eight per cent.

In the whole animal there are many factors whose complex interplay determines cardiovascular function during respiratory acidosis.

It is reported that carbon dioxide exerts both a central and a peripheral action. Under normal circumstances the central effects predominate and are due to direct stimulation of the vasomotor centers; consequently, when carbon dioxide is inhaled vasoconstriction occurs in all sympathetically innervated peripheral vessels. The blood pressure rises, the heart rate is accelerated and the cardiac output is increased (52).

The peripheral action of carbon dioxide tends to oppose these changes by dilating the peripheral vascular bed, particularly the veins



and the capillaries. This dilatation was first demonstrated by von Anrep in 1912 (53) when he perfused the isolated ears of rabbits with Ringer's solution containing different concentrations of carbon dioxide. The systemic effect of such generalized vasodilatation is a fall in blood pressure and it follows therefore that if for any reason the vasomotor impulses are inhibited hypotension will follow the accumulation of carbon dioxide. The importance of vasomotor centers in this respect was first demonstrated by Dale and Evans in 1922 (54) who showed that the rebreathing of expired air would raise the blood pressure in intact cats, but if the brain stem was then destroyed the rebreathing of expired air would be followed by hypotension. This work was confirmed by Pinkston et al (55) in 1936, who showed that the inhalation of carbon dioxide by sympathectomised cats and dogs resulted in a prompt and marked fall in blood pressure.

Much of the work in studying the effect of carbon dioxide on the cardiovascular system has been done using the Diffusion Respiration technique, which has been defined as "gas exchange between the atmosphere and lung alveoli in the absence of rhythmically recurring differences in the barometric pressure of these two areas." (56) This technique has been a favorite tool of research workers because one can study the direct circulatory actions of accumulating carbon dioxide without influence from the secondary effects caused by hyperventilation and changing intrathoracic pressure.

Early in the course of diffusion respiration when the concentration of carbon dioxide is relatively low a transient hypotension may often be



observed (57). This may be due to a direct effect of carbon dioxide on the heart muscle itself. Jerusalem and Starling (33) have shown that concentrations between 12 and 20 per cent carbon dioxide are capable of producing cardiac dilatation and a fall in cardiac output. Later Itami (58) demonstrated that the fall in cardiac output was rapidly succeeded by hypertension and vaso-constriction. Itami found that if he destroyed the spinal cord of the cat from the second dorsal nerve downwards, the administration of carbon dioxide did not produce hypertension, in view of this he concludes that carbon dioxide causes hypertension by increasing the cardiac output and active constriction of the blood vessels from stimulation of the vaso-motor center. Similar conclusions were arrived at by Kaya and Starling (59) who showed that with a spinal cat, the administration of carbon dioxide in the presence of adequate oxygen produces no effect on the general blood pressure. The vaso-constricting effect of carbon dioxide in animals with nerves intact, and its effect in decreasing femoral blood volume flow was demonstrated by Bronk and Gesell in 1927 (60). Later in 1930, Bernthal (61) showed that the administration of ten per cent carbon dioxide to dogs decreased the blood flow in the denervated leg as well as in the innervated leg. Steck and Gellhorn (62) showed that carbon dioxide reduces the blood flow in the hand in normal persons, however this reaction did not occur after sympathectomy.

In 1911 Cannon and Hoskins (63) noted that in asphyxia there is an increase of adrenaline in the blood and a rise in blood pressure. Presumptive evidence for an adrenaline-like response of the cardiovascular system during carbon dioxide breathing has been reported by Itami (58) and



Cathcart and Clark (64). Schaefer et al (65) have presented evidence showing an increased adrenal cortical activity upon administration of 30 per cent carbon dioxide in air and in oxygen. This is indicated in the fall of adrenal cholesterol after exposure to carbon dioxide. More recently, chemical methods of assay have been used and have demonstrated that high concentrations of carbon dioxide (25 per cent) stimulate the release of adrenaline and lesser amounts of noradrenaline (66). The release of catecholamines during diffusion respiration was also reported by Tenney (67), who observed the isotonic contraction of the denervated nictitating membrane of the cat during diffusion respiration. Removal of both adrenal glands decreased by 50 per cent the assayed adrenaline titer. Since only 50 per cent of the response was abolished, one must consider extramedullary chromaffin tissue as well as sympathetic nerves implicated in liberating the remaining catecholamines. Most recently Miller (12) has presented evidence showing that plasma adrenaline and noradrenaline concentrations, estimated by the trihydroxyindole method, progressively rise during diffusion respiration in the dog. Noradrenaline rises predominantly in the first 30 minutes, however adrenaline increases greatly, reaching levels up to  $30 \mu\text{g/L}$  after 60 minutes of apnea.

In brief, acids and carbon dioxide depress isolated heart preparations, however in the intact animal carbon dioxide causes an increase in cardiac output and stimulates sympathetically innervated structures. The peripheral action of carbon dioxide opposes the central effects by dilating certain vessels. Therefore the effect of carbon dioxide on the cardiovascular system depends on which of these two opposing effects predominate.



#### 4. Depression of Catecholamine Activity During Acidosis

In 1920 Snyder and Campbell (1) noticed in perfusing frog's vessels that the adrenaline effect was diminished when the Ringer's perfusion fluid was lowered to pH 7.0. In the same year Snyder and Andrus (2) showed a depression of the effect of adrenaline on the isolated heart of the terrapin by increasing the hydrogen-ion concentration of the perfusate. Recording blood pressure from the carotid artery of dogs, Collip (3) reported that the pressor effect of adrenaline is decreased by raising the hydrogen-ion concentration of the blood by intravenous injection of acid sodium phosphate. From perfusion studies of the frog's heart with Ringer's solution, Salent and Johnston (4) showed that as the hydrogen-ion concentration of the perfusate was increased adrenaline became less effective.

Andrus (5) reported that the action of adrenaline upon the isolated rabbit auricle was reduced when the surrounding Locke's solution was made acidic. The alterations in pH were performed by bubbling a mixture of oxygen and carbon dioxide through the solution. Andrus believed that the adrenaline depression may be due to either of two causes, the change in pH might act on adrenaline itself or it might act by altering the susceptibility of the tissue. In view of this he performed a series of experiments using the more stable tyramine HCl instead of adrenaline. The results with tyramine were similar to those with adrenaline.

Burget and Visscher (6) reported a decrease in the blood pressure response to injected adrenaline in pithed cats, when the blood pH was lowered by reducing the ventilation of the animal. Similarly, a marked decrease in the pressor action of adrenaline was shown by



Stavraky (7) in spinal cats during hypoventilation. Page and Olmstead (8) reported that dogs made acidotic by inhalation of carbon dioxide-oxygen mixtures are refractory to injected adrenaline. Gas mixtures high in nitrogen failed to effect the vasopressor action of adrenaline. Houle et al (9) found that when adrenaline and noradrenaline were administered to dogs during respiratory acidosis, the pressor responses were less than responses obtained in the same animals when the arterial blood  $p^H$  was at or near normal values. Tobian et al (10) reported that noradrenaline induced contractions of isolated arterial smooth muscle is reduced when the bathing solution is made acidic by means of bubbling carbon dioxide through it. Most recently, Nash and Heath (11) have demonstrated that in the intact anaesthetized dog, the peripheral vascular responses of the carotid area to intra-arterial doses of adrenaline and noradrenaline are depressed during hypercapnia and low arterial blood  $p^H$ .

In summary, catecholamine activity is decreased in both isolated tissues and intact animals when the  $p^H$  of the media or blood is lowered by the addition of acid or carbon dioxide.



## 5. Pharmacological Actions of Reserpine and its Influence on Noradrenaline and Tyramine

In 1952, Müller et al (68) isolated reserpine in crystalline form from the root of *Rauwolfia serpentina*. Reserpine is characterized by a variety of Pharmacological effects that vary from species to species in their degree. Some of the typical effects are tranquilization, hypotension, bradycardia, myosis, ptosis, drop of body temperature and increased gastro-intestinal motility. Two features are common to all the effects. The first is the onset of action, which occurs after a certain latency period that can be somewhat shortened by the increase of the dose but cannot be eliminated entirely even when given by intra-arterial injection. The second feature is an exceptionally long duration of action after a single dose. (69)

There is still some controversy as to the mode of action of reserpine. Some investigators feel that the main site of action of reserpine is in the hypothalamic area, and that most of the effects of the drug can be explained on the basis of a reduced central sympathetic activity. This decrease in central sympathetic activity may not be due to a direct suppression since Schneider (70) found that reserpine did not decrease the pressor response evoked by electrical stimulation of vasopressor areas in the brain stem. In view of this, Schneider feels that the decrease in central sympathetic activity after reserpine is probably due to a blockade between afferent and efferent neurons.

Some investigators feel that the central action of reserpine is mediated through release of serotonin and that serotonin plays some



role in regulation of normal brain function. In support of this view, Fletscher et al (71) have reported that reserpine releases serotonin from intestinal tract, platelets and the brain. Holzbauer and Vogt (72) demonstrated that injections of 0.4 mg/Kg. reserpine in cats will deplete the hypothalamus of its noradrenaline content. In later work Muscholl and Vogt (73) demonstrated that in rabbits, cats and dogs treated with reserpine, the peripheral sympathetic tissues lose much of their noradrenaline content.

Several authors feel that in addition to its central action, reserpine also has a direct vasodilator effect on blood vessels. In limb perfusion experiments with cats, McQueen et al (74) showed that injection of reserpine into the perfusion cannula causes an immediate fall in pressure. More evidence for a peripheral action of reserpine was presented by Maxwell et al (75,76). These authors reported that in spinal dogs reserpine produces transient pressor responses even in the presence of ganglionic blocking agents.

The observations of Maxwell et al become more interesting in view of Burn and Rand's (77) observations that when rabbits and dogs are treated with reserpine the noradrenaline-like substance demonstrated by Schmitterlow in 1948 to be present in the wall of the aorta disappears. The pressor response noted by Maxwell et al may have been due to a sudden release of catecholamines from the storage sites.

In summary, it appears that reserpine has a central and a peripheral action. There is no general agreement as to the mode of action at the central level, however at the periphery it appears that reserpine causes a release of catecholamines from the storage sites.



Noradrenaline was first synthesized by Stolz in 1904, who also noted that it is as active in raising the blood pressure of animals as adrenaline. (78) The properties of the synthetic form were first described in 1910 by Barger and Dale (79) who also recognized its sympathomimetic action. The fact that noradrenaline is a naturally occurring substance of biological significance did not receive interest until von Euler pointed out in 1946 that it is present in extracts from heart and splenic tissue. The following year Holtz demonstrated that noradrenaline is present in urine and suprarenal extracts, while Gaddum and Goodwin showed that electrical stimulation of sympathetic nerves liberate noradrenaline. In 1951 von Euler found that noradrenaline is present in nerves containing adrenergic fibers. (78). Swan (80) demonstrated that the main effect of noradrenaline infusion in man is a peripheral vasoconstriction and a rise in both systolic and diastolic blood pressures. Collier et al (81) reported that noradrenaline in dogs as in man decreases or leaves unchanged the cardiac output. Cobbold and Vass (82) showed that noradrenaline exerts a vasoconstrictor action on the skeletal muscle blood vessels of the cat. Burn and Hutcheon (83) reported that the only parts of the vascular bed in animals which have been found to respond to noradrenaline with vasodilatation are the coronary arteries and possibly the intestinal vessels.

The mechanism of action of noradrenaline is still a mystery. It is believed that it acts by catalyzing biochemical processes after having combined with the effector cell by some kind of receptor. There is evidence for two types of adrenergic receptors; a "motor" type (alpha) and an "inhibitory" type (beta) (84,85). However the nature of these



hypothetical structures or systems is unknown. Experimental work indicates that noradrenaline has very little or no affinity for beta receptors (86).

The sympathomimetic action of tyramine was reported in 1910 by Barger and Dale (79).

Our knowledge regarding the mechanism of action of tyramine is due largely to the work of Burn and Rand. In 1932 Burn (87) noticed that the pressor action of tyramine was enhanced after injection of adrenaline. Carlson et al (88) found that in reserpinized cats tyramine failed to cause the usual increase in blood pressure. More recently, Burn and Rand (16) showed that tyramine regains its pressor activity in the reserpinized cat after an infusion of noradrenaline. From this observation Burn and Rand conclude that tyramine normally acts by releasing noradrenaline or adrenaline or both from vascular tissue stores.



## 6. Action of Pitressin on Smooth Muscle

Oliver and Schafer (89) in 1895 were the first ones to note a marked rise of blood pressure in the anesthetized dog upon injection of posterior pituitary extract. The rise in pressure was much more sustained than that obtained with adrenaline. Pitressin, which is one constituent of posterior pituitary extract, has a powerful constrictor effect on capillaries and arterioles. The action is a direct one on the contractile elements since it is not abolished by either adrenergic blockade or vascular denervation. Pitressin causes coronary vasoconstriction in both the isolated and intact heart, and this in turn is thought to depress the heart because of a diminished blood supply (52).



## EXPERIMENTAL METHODS

### Series I. Dog Experiments

Mongrel dogs varying in weight from 7-22 Kgm. were anesthetized with 35 mg/Kgm. intravenous doses of sodium thiopental. A trachial cannula was inserted, and the left jugular vein was cannulated for administration of heparin and anesthetic when required. The femoral artery was cannulated at both the proximal and distal ends of the severed vessel so that blood could be diverted through a flowmeter and returned to the artery. Blood flow in the artery was measured by use of a recording bubble flowmeter, of the type described by Nash and Milligan (90) and recorded on a Grass oscillograph. The blood pressure was taken from a T opening in the flowmeter circuit and measured and recorded by use of a strain gauge and the Grass oscillograph. Peripheral resistance in the femoral arteries was calculated from the pressure to flow ratio and expressed as peripheral resistance units (PRU)(91).

Heparin, 4 mg./Kgm. was used to prevent blood clotting. The original dose was supplemented by 1 mg./Kgm. every 30 minutes. One-half of the animals were reserpinized by intraperitoneal administration of 0.5 mg./Kgm. doses of reserpine for two to six days.

The responsiveness of the vascular bed was determined by measuring the changes in resistance induced by intra-arterial doses of noradrenaline 0.05  $\mu$ g/Kgm., tyramine 10  $\mu$ g/Kgm. and pitressin 0.01 unit /Kgm. These changes were expressed as a percentage change from two minute basic periods taken immediately before the drugs were administered. The percentage change in resistance was calculated



using the following formula,

$$\frac{\text{Response} - \text{Basic}}{\text{Basic}} \times 100$$

The doses were injected through a rubber-stoppered side arm on the flow-meter circuit. These drugs were dissolved in isotonic saline containing 0.05 per cent sodium metabisulphite to act as a preservative. In each case, the concentration of the solution was such that the volume of the dose of each drug was 0.01 ml./Kgm.

The animals were made hypercapnic by breathing 30 per cent carbon dioxide in oxygen. The test drugs were not administered until the blood  $pH$  was 7.1 or lower. Arterial blood was withdrawn through the rubber-stoppered side arm on the flowmeter circuit and the  $pH$  determined either with the Beckman  $pH$  meter, Model GS or the Metrohm Model E322.

In one group of experiments, tolazoline, an adrenergic blocking agent was administered to dogs before hypercapnia to determine whether it would block the usual increase in peripheral vascular resistance observed during hypercapnia. Conversely, in another group of experiments, reserpine was given to dogs in an effort to determine whether it would potentiate the increase in peripheral resistance during hypercapnia.

## Series II. Cat experiments

Cats of both sexes varying in weight from 2.3 to 4.2 Kgm. were used. The animals were anesthetized with intraperitoneal 35 mg./Kg. doses of sodium thiopental and were spinalized by the procedure described by J. H. Burn (92). The animal's respiration was maintained with a Phipps and Bird respirator using a water value of 10 cm. water pressure to avoid excess pressures. The left jugular vein was cannulated for



administration of heparin. One hind limb of the spinal animal was perfused through the femoral artery with its own blood taken from the left carotid artery. Blood flow was kept constant at 4.0 ml./min. by use of a Sigmamotor pump, so that any change in vascular resistance of the hind limb was reflected as a change in perfusion pressure. Systemic blood pressure from the carotid artery and perfusion pressure from a side arm in the perfusion circuit, were measured and recorded by use of strain gauges and the Grass oscillograph.

Heparin, 4 mg./Kgm. was used to prevent blood clotting. The original dose was supplemented by 1 mg./Kgm. every 30 minutes. One-half of the animals were reserpinized by intraperitoneal administration of 2 mg./Kgm. doses of reserpine for two days. Intra-arterial doses of noradrenaline, tyramine and pitressin were administered through a rubber-stoppered side arm in the perfusion circuit. Dose-response relationship of noradrenaline was plotted using the method of Lineweaver and Burk. (93)

The animals were made hypercapnic by breathing 30 per cent carbon dioxide in oxygen. Arterial blood  $pH$  was determined by using the Metrohm E 322  $pH$  meter.

In both series, tyramine was used as a test for the degree of depletion of noradrenaline stores by reserpine pretreatment, and pitressin was used as a test for the responsiveness of the vascular smooth muscle.

In both series of experiments the Fischer "t" test was used to determine the significance of difference between mean values. A P value of 0.05 or less is considered to be significant (95).



Addendum.

Folkow has reported blood flows of 6.5 ml./min./100 Gm. in the hind limbs of cats. (Acta phys. Scandinav. Vol. 27).

In the work presented in this thesis a blood flow of 4 ml./min. was used to perfuse the hind limbs in the cat series.

Preliminary experiments showed that flow rates in the order of 5-10 ml./min. produced extremely high perfusion pressures. A combination of the effects of the pressor drugs and perfusion pressure resulted in pressures beyond the limits of the recording apparatus. In order to circumvent this problem, it was necessary to reduce the blood flow to 4 ml./min. which reduced the perfusion pressure to within practical limits (20-30 mm. Hg.).



#### IV. RESULTS





TABLE I

PRESSURE, FLOW AND RESISTANCE IN HIND LIMBS OF UNTREATED AND  
RESERPINE PRETREATED DOGS BEFORE AND DURING HYPERCAPNIA

UNTREATED				RESERPINE PRETREATED				
	Pressure	Flow	Resistance	Readings/	Pressure	Flow	Resistance	Readings/
	mm.Hg. $\pm$ S.E.	ml/min $\pm$ S.E.	(P/F) $\pm$ S.E.	animals	mm.Hg. $\pm$ S.E.	ml/min $\pm$ S.E.	(P/F) $\pm$ S.E.	Animals
Before	98 $\pm$ 8.5	17.3 $\pm$ 1.8	7.0 $\pm$ 1.1	16/8	86 $\pm$ 7.9	19.5 $\pm$ 1.7	4.6 $\pm$ 0.4	11/6
During	92 $\pm$ 8.2	13.1 $\pm$ 1.5	8.2 $\pm$ 0.9	16/8	69 $\pm$ 6.4	10.7 $\pm$ 1.5	7.4 $\pm$ 0.9	11/6
P value *								
Before and during	0.557	0.107	0.494		0.073	0.003	0.031	

\*Significance of the differences between mean values before and those during hypercapnia.

## EXPERIMENTAL RESULTS

Table I. A summary of data in Appendix 1 and 2, comparing the peripheral vascular responses of untreated and reserpine pretreated dogs to hypercapnia.





TABLE II

A Comparison of the Percentage Elevation of Peripheral Resistance Caused by the Test Drugs in Untreated and Reserpine Pretreated Dogs.

Test Drugs	UNTREATED		RESERPINE TREATED	
	Before CO <sub>2</sub>	During CO <sub>2</sub> Difference*	Before CO <sub>2</sub>	During CO <sub>2</sub> Difference*
0.05 µg/kg Noradrenaline, I.A.				
Mean Response (P.R.U.)	142±18	80±8 P 0.04	433±88	162±35 P 0.01
Tests/Animals	16/8	16/8	11/6	11/6
Difference**		P 0.01		
10 µg/kg Tyramine, I.A.				
Mean Response (P.R.U.)	175±29	178±23 P 0.07	682±133	365±91 P 0.05
Tests/Animals	10/5	10/5	9/5	9/5
Difference**		P 0.01		
0.01 units/kg Pitressin, I.A.				
Mean Response (P.R.U.)	75±29	59±25 P 0.11	232±56	175±34 P 0.17
Tests/Animals	10/5	10/5	9/6	9/6
Difference**		P 0.02		

\*Significance of the differences between the mean responses to the test drugs before and during hypercapnia.

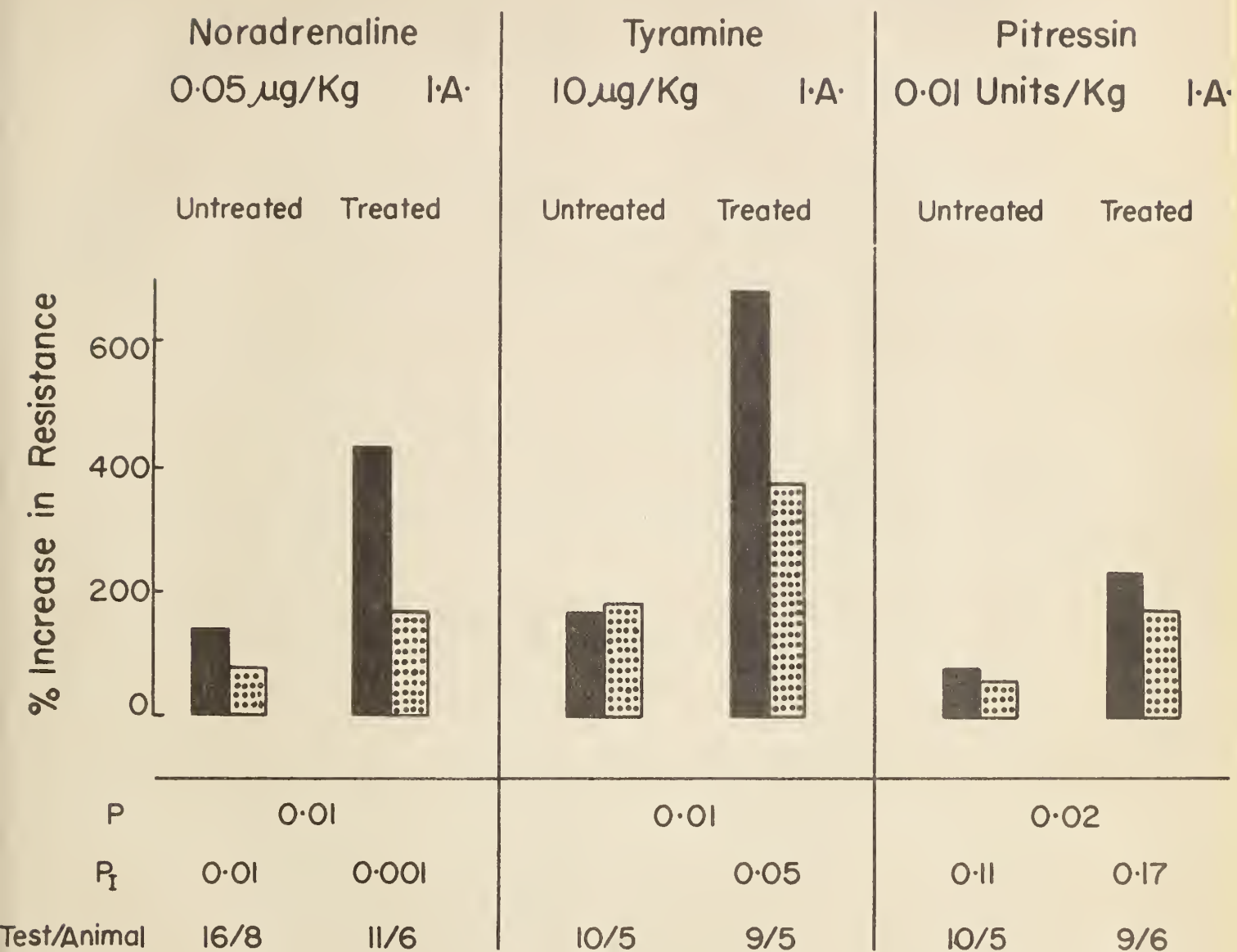
\*\*Significance of the differences between the mean responses of untreated and reserpine treated animals to the test drugs, before hypercapnia.

Table II. The mean percentage elevation of peripheral resistance in untreated and reserpine pretreated dogs, which was caused by test doses of noradrenaline, tyramine and pitressin administered before and after hypercapnia. Supporting data appear in Appendices 1 and 2. These data are represented in histogram form in Figure 1.



Figure 1.

# Peripheral Vascular Responses of Reserpine Treated and Untreated Dogs Before and During Hypercapnia.



## Legend:



- Before Hypercapnia

- During Hypercapnia

P - Reserpine treated and untreated

P<sub>I</sub> - Before and during Hypercapnia





TABLE III

The Effect of Adrenergic Blockade on the Peripheral Vascular Responses of Dogs to Hypercapnia.

	UNTREATED			AFTER TOLAZOLINE		
	Before CO <sub>2</sub>	During CO <sub>2</sub>	Difference*	Before CO <sub>2</sub>	During CO <sub>2</sub>	Difference*
Mean Resistance (PRU)	3.1±0.71	6.4±1.2	3.3	5.5±0.71	5.8±0.71	0.3
Animals	4	4	P 0.03	4	4	P 0.557
Mean pH	7.20	6.91		7.25	6.91	
Range	7.02-7.43	6.80-7.11		7.05-7.47	6.81-7.05	
Difference**	3.1	vs	5.5	P 0.003		
Difference***	3.3	vs	0.3	P 0.006		

\*Significance of the difference between mean peripheral resistance before and during hypercapnia.

\*\*Significance of the difference between the vascular resistances before and after Tolazoline.

\*\*\*Significance of the difference between the mean change in peripheral resistance caused by hypercapnia before and after Tolazoline.

Table III. A comparison of the peripheral vascular responses of dogs to hypercapnia before and after adrenergic blockade induced by an intravenous dose of tolazoline (2.5 mg/kg). Supporting data appear in Appendix 3, including responses to noradrenaline before and after tolazoline as evidence of the degree of adrenergic blockade.





TABLE IV

The Effect of Reserpine on the Peripheral Vascular Responses of Dogs to Hypercapnia.

	UNTREATED			AFTER RESERPINE		
	Before CO <sub>2</sub>	During CO <sub>2</sub>	Difference*	Before CO <sub>2</sub>	During CO <sub>2</sub>	Difference*
Mean Resistance (P.R.U.)	3.4±0.25	10.4±2.7	7	4.4±0.43	9.5±1.2	5.1
Animals	4	4	P 0.02	4	4	P 0.002
Mean pH	7.21	6.72		7.12	6.78	
Range	7.05-7.40	6.57-6.87		7.00-7.42	6.70-6.96	
Difference**	3.4 vs 4.4	P 0.064				
Difference***	7 vs 5.1	P 0.495				

\*Significance of the difference between mean peripheral resistance before and during hypercapnia.

\*\*Significance of the difference between the vascular resistances before and after Reserpine.

\*\*\*Significance of the difference between the mean change in peripheral resistance caused by hypercapnia before and after reserpine.

Table IV. A comparison of the peripheral vascular responses of dogs to hypercapnia before and after intravenous doses of reserpine (1.5 mg/kg). Supporting data appear in Appendix 4, including responses to tyramine before and after reserpine as an indication of the degree of potentiation of the action of a drug which is, presumably, a noradrenaline releasing agent.





Figure 2.

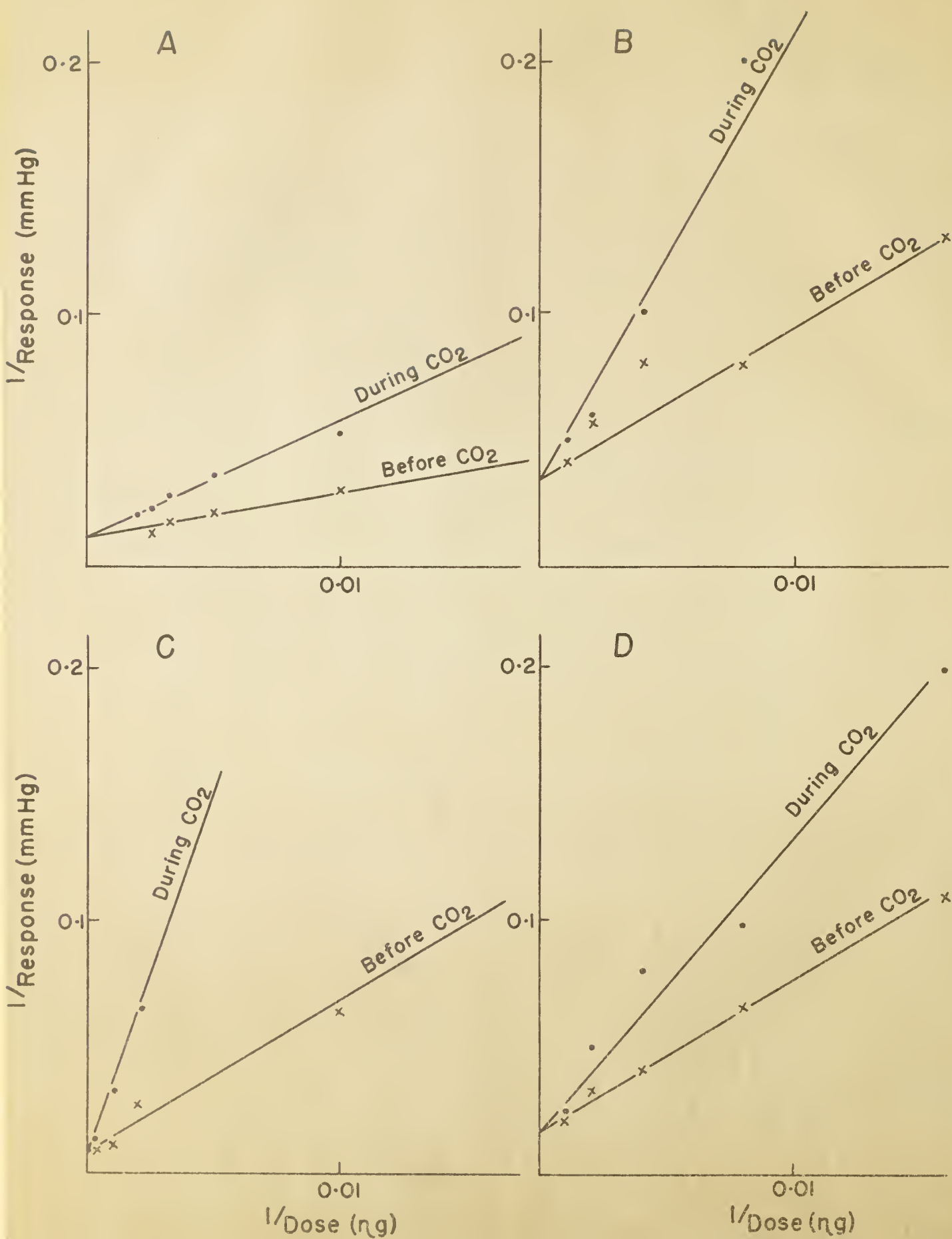


TABLE V

THEORETICAL MAXIMUM RESPONSES TO I.A. DOSES OF NORADRENALINE  
BEFORE AND DURING CARBON DIOXIDE IN UNTREATED SPINAL CATS

Graph	Wt.kg.	pH	BEFORE CO <sub>2</sub>		pH	DURING CO <sub>2</sub>		
			Slope	Intercept R max. mm/Hg		Slope	Intercept R max. mm/Hg	Slope change
A	3.2	7.25	0.25	125	6.60	0.57	125	+
B	3.6	7.30	0.60	30	6.60	2.0	30	+
C	2.5	7.25	0.57	165	6.55	3.0	165	+
D	3.1	7.35	0.60	65	6.65	1.2	65	+

Figure 2 shows the data in Appendix 5 represented in the manner of the Lineweaver and Burk plot for estimation of maximum effect. Table V contains the slopes and the theoretical maximum responses of the data in graphs A to D.





Figure 3.

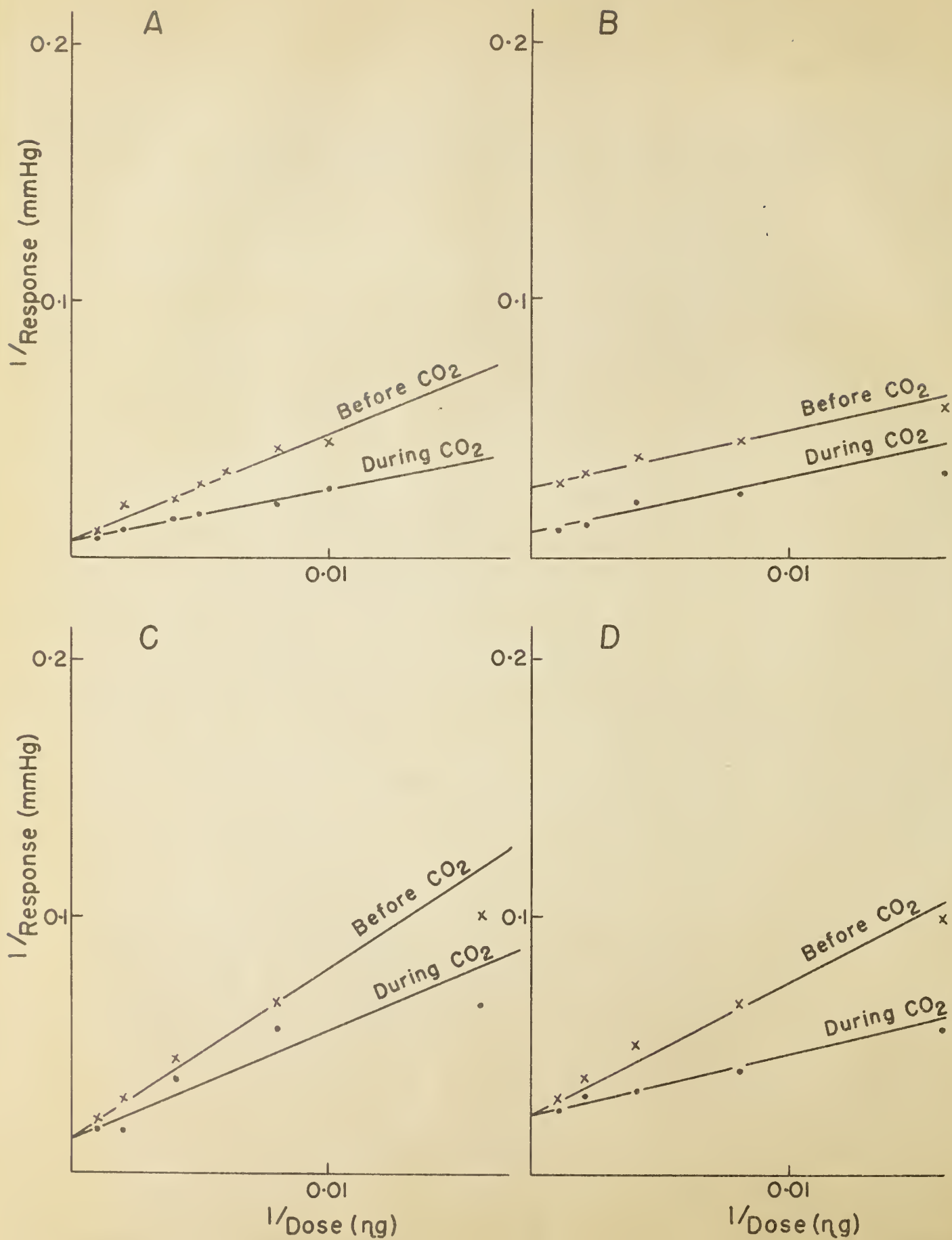


TABLE VI

THEORETICAL MAXIMUM RESPONSES TO I.A. DOSES OF NORADRENALINE  
BEFORE AND DURING CARBON DIOXIDE IN RESERPINE  
PRETREATED SPINAL CATS

Graph	Wt.kg.	pH	BEFORE CO <sub>2</sub>			pH	DURING CO <sub>2</sub>		
			Slope	Intercept R max. mm/Hg			Slope	Intercept R max. mm/Hg	Slope change
A	2.6	7.30	0.44	165		6.70	0.20	165	—
B	2.3	7.40	0.22	40		6.64	0.16	90	—
C	3.6	7.15	0.62	60		6.47	0.50	60	—
D	3.6	7.38	0.50	45		6.50	0.21	45	—

Figure 3 shows the data in Appendix 6 plotted in the manner of the Lineweaver and Burk method. The above table contains the slopes and the theoretical maximum responses of the data in graphs A to D.



TABLE VII

Pressor Responses to 40  $\mu$ g Tyramine, I.A. in Untreated Cats Before and During CO<sub>2</sub>.

BEFORE CO <sub>2</sub>			DURING CO <sub>2</sub>	
Wt.kg.	pH	Response mm/Hg	pH	Response mm/Hg
3.2	7.25	21.2	6.60	31.2
3.6	7.23	17.5	6.45	80.0
3.1	7.35	15.0	6.65	37.5
3.6	7.30	10.0	6.60	35.0
Mean Response		15.9±2.3	45.9±11.4	
Significance of the difference between mean response before and during CO <sub>2</sub> . P = 0.041				

TABLE VIII

Pressor Responses to 40  $\mu$ g Tyramine, I.A. in Reserpine Pretreated Cats Before and During CO<sub>2</sub>.

BEFORE CO <sub>2</sub>			DURING CO <sub>2</sub>	
Wt.kg.	pH	Response mm/Hg	pH	Response mm/Hg
2.6	7.30	7.5	6.70	25.0
2.3	7.40	5.0	6.64	10.0
3.6	7.15	7.5	6.47	5.0
3.6	7.38	5.0	6.50	6.2
Mean Response		6.25±0.7	11.6±4.6	
Significance of the difference between mean response before and during CO <sub>2</sub> . P = 0.308				



TABLE IX

Pressor Responses to 0.1 unit Pitressin, I.A. in Untreated Cats Before and During CO<sub>2</sub>.

BEFORE CO <sub>2</sub>			DURING CO <sub>2</sub>	
Wt.kg.	pH	Response mm/Hg	pH	Response mm/Hg
3.6	7.23	25	6.45	0
3.6	7.30	7.5	6.60	0
Mean Response		16.25		0

TABLE X

Pressor Responses to 0.1 unit Pitressin, I.A. in Reserpine Pretreated Cats Before and During CO<sub>2</sub>.

BEFORE CO <sub>2</sub>			DURING CO <sub>2</sub>	
Wt.kg.	pH	Response mm/Hg	pH	Response mm/Hg
2.6	7.30	65	6.70	27.5
2.3	7.40	15	6.64	5.0
3.6	7.15	22.5	6.47	5.0
3.6	7.38	30.0	6.50	7.5
Mean Response		33.1±11.1		11.2±5.4
Significance of the difference between mean response before and during CO <sub>2</sub> . P = 0.133				



## V. DISCUSSION

### Series I

In the first series of experiments (see Table II and Fig. 1) the untreated dogs showed the expected hypercapnic depression of the noradrenaline response but reserpine treated animals not only showed a significant increase in their response but also a significant depression during hypercapnia. This is contrary to the first postulate that the depression is due to partial saturation of the receptors by noradrenaline released during hypercapnia. The data suggest that the depression is not affected at the receptor level.

The response to tyramine was not depressed by hypercapnia in the untreated dogs, which suggests that the hypercapnia did potentiate the release of noradrenaline and thus overcome the hypercapnic depression. This is in partial agreement with the second postulate that the hypercapnia may, like intravenous reserpine, potentiate the action of tyramine. However, up to six days pretreatment with reserpine did not abolish the peripheral vascular response to tyramine. In fact, it is significantly increased and contrary to the second postulate the reserpine treated animals showed hypercapnic depression. Thus, either dogs are different from rats, rabbits and cats and are not easily depleted of their vascular noradrenaline or, tyramine does not act solely by release of noradrenaline.

In both untreated and reserpine pretreated dogs the mean responses to pitressin were slightly, but not significantly, decreased during hypercapnia. These data suggest that the hypercapnic depression is



effected at the contractile mechanism rather than at the receptor site. Contrary to expectations, the responses to pitressin were potentiated by reserpine, which also suggests reserpine acts beyond the receptor mechanism.

The hypercapnic depression of the responses to noradrenaline and pitressin<sup>in</sup> both untreated and reserpine pretreated dogs, suggest that these depressions are probably effected at the contractile mechanism rather than at the receptor mechanism of the vascular smooth muscle.

This investigation is based partially on Miller's (12) report that hypercapnia is accompanied by an increase in circulating catecholamines. If the catecholamines were released from vascular tissue, which can be depleted of these amines by treatment with reserpine, it might reasonably be expected that pretreatment with reserpine would abolish the elevation of peripheral resistance which accompanies hypercapnia. However, from Table I it will be noted that both untreated and reserpine pretreated dogs showed an elevation of peripheral resistance during hypercapnia. In view of contradictory results and failure to support the postulates it was decided to test the effects of intravenous reserpine and of an adrenergic blockade with tolazoline on the hypercapnic elevation of peripheral resistance. If this elevation is due to an increase in the concentration of catecholamines it might be expected that adrenergic blockade would abolish the effect but intravenous reserpine would potentiate it, as it potentiates tyramine (14).

After adrenergic blockade, as indicated in Table III, the



increase in peripheral resistance during hypercapnia is very slight and statistically insignificant. This supports the thesis that the increased resistance is active, resulting from release of catecholamines. The data in Table IV show that intravenous reserpine increases the mean peripheral resistance from 3.4 to 4.4 P.R.U. During hypercapnia the increase in resistance (5.1 P.R.U.) is not statistically different from that occurring in the untreated dogs (7.0 P.R.U.). Because the basic resistance was increased by the reserpine, no valid information regarding the reserpine potentiation of catecholamine release during hypercapnia can be drawn from this group of experiments.

## Series II

In view of the inconclusive results in Series I it was decided to do a second series of experiments using cats instead of dogs and a constant flow instead of a variable flow method. In addition, in order to attempt to eliminate variables which might be introduced by an anaesthetic or central nervous system effects, the cats were spinalized.

The use of a flowmeter in the study of vascular resistance has been questioned, since the quantity of blood flowing through an organ is dependent on both the blood pressure and the vessel tone. The estimated parameter, resistance, is a resultant of two measurements, pressure and flow, the latter being dependent upon the former. Flow may increase as a result of an increased pressure head but also as a result of distending the elastic vessels. Thus, when pressure is varying it is impossible to distinguish active from passive changes in resistance. Perfusion pumps cause damage to blood cells as blood passes through them. This trauma is believed to cause the liberation of



5-hydroxytryptamine and ATP from the blood cells, and thus either increasing or decreasing the tone of the perfused vessels (86,94). This technique however affords the advantage that perfusion pressure varies directly with resistance changes so that any changes in pressure reflect active changes in resistance.

According to Clark's theories (86), response is directly proportional to the number of receptors occupied by the stimulating drug. In this case the maximum response to injected noradrenaline might be expected to decrease during hypercapnia which presumably causes release of noradrenaline and thus partial saturation of the receptors. After reserpinization which depletes the stores of available noradrenaline for release by carbon dioxide, the number of available receptor sites would be increased. In that event if noradrenaline depression is mediated at the receptor level no change in maximum response should be expected during hypercapnia.

The experiments with cats were designed to obtain maximum responses before and during carbon dioxide in untreated and reserpine pretreated animals. Early experiments showed that the expected rectangular hyperbolic curves of dose-response plots did not plateau so that accurate estimations of the maximum response could not be made. In addition, at high intra-arterial doses the systemic pressure also rose, indicating part of the drug was passing through the femoral area into the general circulation. Because of these experimental difficulties, it was decided to use smaller doses and to estimate the theoretical maximum responses using the Lineweaver and Burke conventions.

A. J. Clark (86) and his co-workers were among the first to use the concept of drug-receptor interaction in interpreting the relation



between drug concentration and response. Clark, in attempting to derive an equation relating the two variables, made the following assumptions; the reaction between an active drug and its receptor is reversible and obeys the law of mass action; that the receptors are all uniform in their affinity for the drug and that the magnitude of the response is directly proportional to the fraction of the total number of receptors combined with the drug.

Clark's equation related action to drug concentration as follows:

$$A = \frac{A_m (D)}{K_D + (D)}$$

where A is the measured action,  $A_m$  is the maximal action when all receptors are occupied, (D) is the concentration of added drug, and  $K_D$  is the dissociation constant of the drug-receptor complex. This equation is analogous to the equation relating velocity of an enzyme reaction to concentration of substrate:

$$v = \frac{V \cdot s}{K_m + s}$$

where v is the velocity when the substrate concentration is s, V is the maximum velocity obtained when the substrate concentration is high enough to saturate the enzyme, and  $K_m$  is a constant of the enzyme for this substrate. One of the methods used to plot this relationship is the one introduced by Lineweaver and Burk (93),  $1/v$  is plotted against  $1/s$ . The graph cuts the vertical axis at a point which gives  $1/V$  and has a slope of  $K_m/V$ . Applying this method to Clark's equation,  $1/A$  is plotted against  $1/D$ , a straight line results with an intercept equal to  $1/A_m$  and a slope equal to  $K_D/A_m$ . The dose-response curves from the cat series were plotted as reciprocals in the manner of Lineweaver and Burk (Fig. 2 and 3). The plots of results of untreated and



reserpine pretreated animals before and during hypercapnia resemble the curves for competitive inhibition of an enzyme reaction. That is, in all cases with only one exception in the reserpine treated animals (Fig. 3B), the theoretical maximum response is the same before and during carbon dioxide. In the case of untreated animals the slope increased as the response to low doses were depressed during hypercapnia. However, in the reserpine pretreated cats the responses were potentiated during hypercapnia and the slope decreased. This last observation is contrary to the results in the dog series.

The pressor responses to tyramine in the untreated cats (Table VII) were greatly potentiated during hypercapnia. This observation is qualitatively similar to the results in the dog experiments, although in those cases the increases in responses were slight. In the reserpine pretreated cats (Table VIII) the responses during hypercapnia were also slightly increased in most cases, although not to the extent observed in the untreated cats. This is again contrary to the results in the dog series, where a decrease in response was noted during hypercapnia in the reserpinized dogs.

The pressor responses to pitressin (Tables IX and X) were depressed by hypercapnia in all of the cases in both untreated and reserpine pretreated cats. This is in agreement with the observations in the dog series.

From a consideration of all the data in both series it appears that the depression of noradrenaline response during hypercapnia is mediated at the level of the contractile mechanism rather than at the receptor mechanism. This however leaves unanswered the apparent competitive



inhibition indicated in the cat series. If it is assumed that it is a competitive inhibition, then the question arises regarding the nature of the inhibition. On the other hand this apparent inhibition may be an artifact due to uncontrolled variables against which Furchgott has cautioned.

The assumptions of Clark's hypothesis are still controversial and, when used as the basis to elucidate the mode of action of pharmacological agents, have been supported by some (96,97) and criticized by other workers (86,102,103). Chen and Russel (96) used the graphical methods devised by Lineweaver and Burk in their study of blood pressure responses to cardiovascular drugs and their antagonists in the whole animal. Kirschner and Stone (97) used the concepts of Clark and Lineweaver and Burk in their interpretation of the mode of action of curare at the myoneural junction.

Clark's concept assumes that the "receptors" are enzymes or at least behave like enzymes and most of the objections against Clark's hypothesis appear to be based on this fact. However, Martin (98) in his textbook on Biological Antagonism states, "There seems no reason to regard the reaction sites on effector cells as being different in any fundamental way from those on enzyme surfaces." Tending to support this view is the work of Brown et al (99) who found that after the administration of dibenzyline the noradrenaline content of venous blood from the colon greatly increased during electrical stimulation of mesenteric nerves. These authors said that the prevention of the inactivation of noradrenaline by an adrenergic blockade suggests an enzyme substrate combination at the receptor sites. Furchgott (100)



in referring to the work of Brown et al noted that other interpretations may explain their results. Furchgott stated that the  $\beta$ -haloalkylamines, of which dibenzylamine is a member, in some manner cause a much greater release of transmitter from the sympathetic nerve endings and that these agents not only block certain adrenergic receptors but also inhibit O-methylating enzyme, which Axelrod maintains is the chief enzyme in the inactivation of the transmitter. Recently Belleau (101) compared the activity of ( - ) noradrenaline labelled with deuterium on the  $\alpha$  - carbon with the activity of non-labelled ( - ) noradrenaline, using the cat's nictitating membrane as the test organ. No difference in the magnitude of the contraction or duration of the response was noted. In view of the stereospecificity of enzymes, Belleau concludes that this evidence proves that noradrenaline is not a substrate for the receptor.

Another point in Clark's hypothesis which has been questioned is that the percentage of receptors occupied is equal to the percentage response of the tissue. Stephenson (102) from his work with acetylcholine and alkyltrimethylammonium salts contends that response is not linearly proportional to number of receptors occupied since different drugs may have different capacities for initiating response and hence may occupy different proportion of receptors although producing equal responses.

Nickerson (103) does not feel that Clark's hypothesis is reliable since Graham and Lewis (104) concluded that  $\beta$ -haloalkylamines in low doses competitively inhibit adrenaline, while he, in later work, showed that  $\beta$ -haloalkylamines, through alkylation, form a stable bond with the receptor or some adjacent structure, thus competitive inhibition is unlikely.



Paton (105) has recently pointed out that Clark's theory in its<sup>53</sup> original form does not explain why some drugs are antagonists and some stimulants; nor does it account for the existence of partial agonists, which are capable of stimulating, but cannot produce the normal maximum response, nor for the antagonism displayed by such partial agonists to more powerful agonists. Stephenson (102) in an attempt to account for these difficulties has proposed that drugs may have different capacities for initiating a response. Paton (105) has advanced this theory by assuming that excitation by a stimulating drug is proportional to the rate of drug-receptor combination, rather than to the proportion of receptors occupied by the drug. The properties of a drug can then be specified by two rate constants:  $k_1$ , the association rate constant, and  $k_2$ , the dissociation rate constant. The value of  $k_2$  determines whether the drug is a powerful stimulant, a partial agonist or an antagonist.

The application of Clark's equation to perfusion experiments has also been criticized. Furchgott (86) in a review article states, "The complicated architecture of vascular beds and the fact that resistance to flow in small tubes varies inversely as the fourth power of the radius of such tubes make any attempt to apply Clark's equation in perfusion experiments utterly useless." Although this is an extreme point of view, it does emphasize the caution necessary and the difficulty in interpreting data obtained by this method. In addition to the above mentioned difficulties the drug concentration near receptors may not be the same as that in the blood circulation. In a living system, the elimination of a drug from tissues is an unknown factor that introduces



another error in the concentrations used for computation.

The responses to pitressin, in both series, suggest that the contractile mechanism is depressed during hypercapnia and potentiated after reserpine, which suggests that both carbon dioxide and reserpine have their effects on the contractile mechanism of the muscle beyond the adrenergic receptor mechanism.

Any explanation of the mechanism of hypercapnic depression of the vasoconstrictor drugs is still speculative but some recent work of Woolley (106) and of Sears (107) may aid in elucidating the problem. Woolley and Campbell (106) reports that contraction of smooth muscle is due to a reaction between calcium ions ( $\text{Ca}^{++}$ ), actomyosin and adenosine triphosphate (ATP), and that serotonin combines with a lipid receptor substance which aids the passage of  $\text{Ca}^{++}$  across the membrane to initiate a muscle contraction. Sears and Eisenberg (107) have reported that carbon dioxide causes a liberation of hydrated cations from cell membranes which tends to decrease the water miscibility of the membrane and to increase its electrical resistance. These authors suggest that this may explain the hypoexcitability of cells during high carbon dioxide tension by decreasing the ease of ionic penetration.

If the mechanisms of the stimulating actions of noradrenaline and tyramine are similar to those suggested by Woolley for serotonin, the hypercapnic depression may be due to a change in the membrane properties causing a decrease in the ease of passage of calcium ions which could, however, be overcome by increasing the concentration of the stimulants. Such a mechanism would explain the apparent competitive inhibitory action of the carbon dioxide observed in our experiments. However, there remains unexplained the fact that in reserpinized dogs the hypercapnia caused the



expected depression of noradrenaline response while in reserpinized cats it caused a potentiation.

Reserpinization potentiated the responses to noradrenaline, tyramine and pitressin in the dogs. If Woolley's suggestion, that the passage of  $\text{Ca}^{++}$  into the cell, is an essential part of the mechanism of stimulation then it is possible that the effect of reserpine is to aid the passage of  $\text{Ca}^{++}$  ions across the lipid portion of the membrane. If that is the case, then the dehydration of the membrane by carbon dioxide postulated by Sears and Eisenberg would make the lipid more continuous so that in reserpinized animals the action of carbon dioxide may be to make the passage of ions easier rather than more difficult. If this hypothesis was applied to the cat series it would explain the increase in response to noradrenaline during hypercapnia. As was shown in the dose-response plots for the unreserpinized animals, the plots of the experiments with reserpinized animals showed the same theoretical maximum effect during as before hypercapnia. This would be expected if  $\text{Ca}^{++}$  is the limiting factor and the maximum effect was reached when an excess of  $\text{Ca}^{++}$  passed the membrane. Although this hypothesis may explain the action in the cat it leaves unexplained the hypercapnic depression of noradrenaline response in the reserpinized dogs, as well as the depression of the pitressin response during hypercapnia in both the reserpinized and untreated animals.



## VI. SUMMARY AND CONCLUSIONS

### Summary

1. The elevation of peripheral vascular resistance during hypercapnia, in both untreated and reserpine pretreated dogs, can be blocked by adrenergic blockade.

2. In untreated dogs the peripheral vascular responses to noradrenaline and pitressin were depressed during hypercapnia, while the tyramine response was not depressed but appeared to be slightly increased.

3. In reserpine pretreated dogs there were increased responses to noradrenaline, tyramine and pitressin, with a depression of all these responses during hypercapnia.

4. The vascular responses to noradrenaline and pitressin were depressed during hypercapnia in the untreated cats, while the tyramine responses were potentiated. Dose-response curves indicate that the theoretical maximum response to noradrenaline was the same during as before hypercapnia.

5. In reserpine pretreated cats the noradrenaline and tyramine responses were potentiated and the pitressin responses were depressed during hypercapnia.

### Conclusions

1. The elevation of peripheral vascular resistance during hypercapnia can be blocked by adrenergic blockade, which suggests that the elevation is caused by increased blood concentration of catecholamines.



2. The hypercapnic depression of the noradrenaline response 57

appears to be effected beyond the receptor level. This conclusion is supported by the observations of Sears and Eisenberg that carbon dioxide influences membrane permeability.

3. The response to tyramine could not be abolished in reserpine pretreated dogs, which suggests that in dogs tyramine may have a direct stimulating action in addition to its ability to liberate noradrenaline.

4. Potentiation of the pitressin response by reserpinization, suggests that reserpine, in addition to its ability to deplete tissues of their noradrenaline content, has an action beyond the receptor level, possibly on membrane permeability.



## BIBLIOGRAPHY

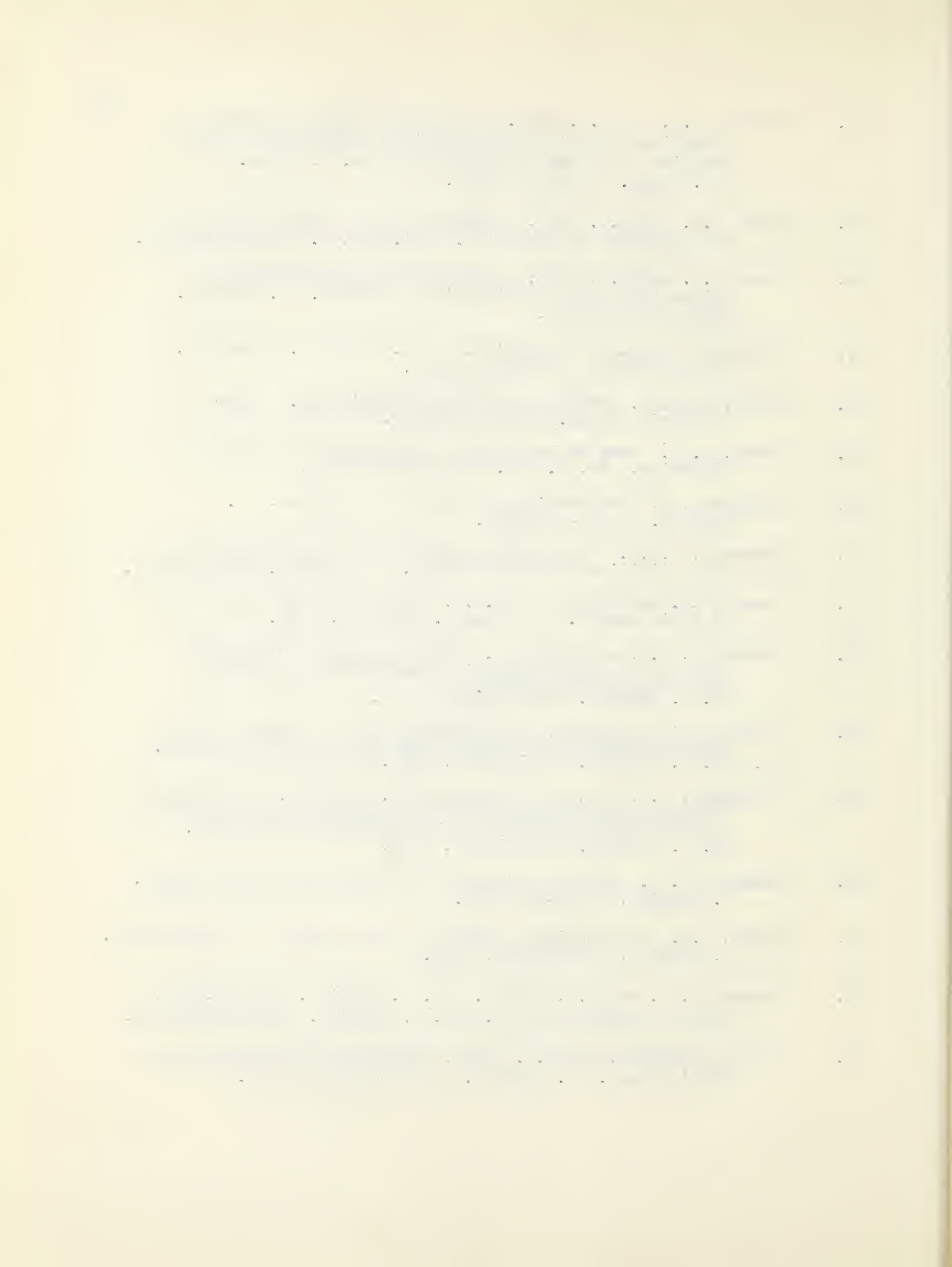


## BIBLIOGRAPHY

1. Snyder, C.D. and W.A. Campbell, Jr. Vascular Reaction to Epinephrin in Perfusates of Various H-ion Concentration. *Am. J. Physiol.* 51: 199-200, 1920.
2. Snyder, C.D. and E.C. Andrus. On The Relation Between Tonus and Smooth Muscle in The Terrapin Heart. *J. Pharm. & Exp. Therap.* 14: 1-24, 1920.
3. Collip, J.B. Reversal of Depressor Action of Small Doses of Adrenalin. *Am. J. Physiol.* 55: 450-454, 1921.
4. Salant, W. and R.L. Johnston. The Response of the Isolated Frog Heart To Changes in Hydrogen-ion Concentration and Adrenaline. *J. Pharm & Exp. Therap.* 23: 373-383, 1924.
5. Andrus, E.C. The Effect of Certain Changes in The Perfusate upon the Isolated Auricles of the Rabbit. *J. Physiol.* 59: 361-372, 1924.
6. Burget, G.E. and M.B. Visscher. Variation of the pH of The Blood and The Response of the Vascular System to Adrenaline. *Am. J. Physiol.* 81: 113-123, 1927.
7. Stavrak, G.W. The Effect of Pulmonary Ventilation on The Pressor Action of Adrenaline. *Am. J. Physiol.* 137: 485-491, 1942.
8. Page, I.H. and F.Olmstead. Influence of respiratory gas mixtures on arterial pressure and Vascular reactivity in "Normal" and hypertensive dogs. *Circulation* 3: 801-819, 1951.
9. Houle, D.B., M.H. Weil, E.B. Brown Jr., and G.S. Campbell. Influence of Respiratory Acidosis on E.C.G. and Pressor Responses to Epinephrine, Norepinephrine and Metorominal. *Proc. Soc. Exper. Biol. & Med.* 94: 561-564, 1957.
10. Tobian, L., S. Martin and W.Eilers. Effect of pH on norepinephrine-induced contractions of isolated arterial smooth muscle. *Am. J. Physiol.* 196: 998-1002, 1959.
11. Nash, C.W. and C. Heath. Vascular Responses to Catecholamines during respiratory changes in pH. *Am. J. Physiol.* 200: 755-758, 1961.
12. Miller, R.A. Plasma Adrenaline and Noradrenaline During Diffusion Respiration. *J. Physiol.* 150: 79-90, 1960.
13. Burn, J.H. and M.J. Rand. Fall of Blood Pressure after a Noradrenaline Infusion and its Treatment by Pressor Agents. *Brit. Med. J.* 1: 394-397, 1959.



14. Gillis, C.N. and C.W. Nash. The initial pressor actions of bretylium tosylate and guanethidine sulphate and their relation to release of catecholamines. *J. Pharm. & Exp. Therap.* In Press, 1961.
15. Burn, J.H. and M.J. Rand. Noradrenaline in Artery Walls and its Dispersal by Reserpine. *Brit. Med. J.* 1: 903-908, 1958.
16. Burn, J.H. and M.J. Rand. The Action of Sympathomimetic Amines in Animals Treated with Reserpine. *J. Physiol.* 144: 314-336, 1958.
17. The ABC of Acid-Base Chemistry. 4 Ed. Horace W. Davenport. The University of Chicago Press.
18. Smith, Homer W. Principles of Renal Physiology. Oxford University Press. New York, 1956.
19. Pitts, R. F. Some Reflections on Mechanisms of Action of Diuretics. *Am. J. Med.* 24: 745-763, 1958.
20. Hemingway, A. The Sensitising Action of Alkalies. *J. Physiol.* 62: 81-87, 1926.
21. McDowall, R.J.S. The Influence of acid-base equilibrium on the activities of Blood Vessels. *J. Physiol.* 65: 25-31, 1928.
22. Evans, C.L. and Underhill, S.W.F. Studies on the Physiology of plain muscle. *J. Physiol.* 58: 1-14, 1923.
23. Furchgott, R.F. and Wales, M. Effect of pH on contractile activity of rabbit intestinal Smooth muscle with and without added Substrates. *Am. J. Physiol.* 167: 386-398, 1951.
24. Fraser, Lois McPhedron. A Comparison of the Effects on the Isolated Beating Intestine of CO<sub>2</sub> and of a Mineral Acid. *Am. J. Physiol.* 72: 119-124, 1925.
25. Oettinger, W.F. von, T. Sollmann and Y. Ishikawa. The Effects of Bicarbonate Buffers and of Carbon Dioxide on the Motor Functions of the Excised Small Intestines of Rabbits. *Am. J. Physiol.* 86: 661-674, 1928.
26. Gaskell, W. H. On the toxicity of the heart and blood vessels. *J. Physiol.* 3: 48-75, 1880.
27. Mines, G.R. On functional analysis by the action of electrolytes. *J. Physiol.* 46: 188-235, 1913.
28. Andrus, E. C. and E.P. Carter. *Am. J. Physiol.* 59: 227, 1922.  
(Cited by Görtler et al.) *Am. J. Physiol.* 146: 478-486, 1946.
29. Daly, I, DeBurgh, and A.J. Clark. The action of ions upon the Frog's Heart. *J. Physiol.* 54: 367-383, 1920-21.



30. Andrus, E.C. and E.P. Carter. The development and propagation of the excitatory process in the perfused heart. Heart 11: 97-107, 1924.
31. Drury, A.N. and E.C. Andrus. The influence of Hydrogen-ion concentration upon conduction in the auricle of the Perfused Mammalian Heart. Heart, 11: 389-403, 1924.
32. Gertler, M.M., H.E. Hoff and D.G. Humm. The Acid tolerance of the dog heart. Am. J. Physiol. 146: 478-486, 1946.
33. Jeruselem, E. and Starling, E.H. On the significance of carbon dioxide for the heart beat. J. Physiol. 40: 279-294, 1910.
34. Whitehorn, W.V. and J.W. Bean. Cardiac changes induced by O<sub>2</sub> at high pressure, CO<sub>2</sub> and Low O<sub>2</sub>, as manifest by the Electrocardiogram. Am. J. Physiol. 168: 528-537, 1952.
35. McElroy, Jr. Wm. T., A.J. Gerdes and E.B. Brown, Jr. Effects of CO<sub>2</sub>, Bicarbonate and pH on the performance of Isolated perfused Guinea Pig Hearts. Am. J. Physiol. 195: 412-416, 1958.
36. Miller, F.A., F.B. Brown, J.J. Buckley, F.H. von Bergen and R.L. Varco. Respiratory acidosis; its relationship to cardiac function and other physiologic mechanisms. Surgery 32: 171, 1952.
37. O'Shaughnessy, L. The vagus and its relation to the surgery of the lung. J. Thorac. Surg., 5: 386-392, 1936.
38. Sloan, H.E. The vagus nerve in Cardiac Arrest; the effect of Hypercapnia, Hyponia and Asphyxia on Reflex Inhibition of the Heart. Surg., Gyn. and Obst. 91: 257-264, 1950.
39. Miller, F.A. et al. Surgical Forum. Philadelphia, 1951. W. B. Saunders Co., p. 35-40.
40. Heath, C. and E.B. Brown, Jr. Posthypercapnic Hemodynamic Changes in Dogs. J. App. Physiol. 8: 495-498, 1956.
41. Fern, W.O. and Cobb, D.M. Evidence for a Potassium shift from plasma to muscles in Response to an increased Carbon dioxide tension. Am. J. Physiol. 112: 41-55, 1935.
42. Young, W.G., W.C. Sealy and J.S. Harris. The Role of Intracellular and Extracellular Electrolytes in the Cardiac Arrhythmias produced by prolonged Hypercapnia. Surgery. 36: 636-649, 1954.
43. Mackay, J.L. Effects of a narcotic level of CO<sub>2</sub> on the plasma potassium and respiration of Cats. Am. J. Physiol. 151: 469-478, 1947.
44. Guest, G.M., B. Mackler and H.C. Knowles. Effects of Acidosis on Insulin action and on Carbohydrate and Mineral Metabolism. Diabetes 1: 276-282, 1952.



45. Graubarth, H., B. Mackler and G.M. Guest. Effects of Acidosis on utilization of glucose in Erythrocytes and Leucocytes. *Am. J. Physiol.* 172: 301-308, 1953.
46. Markwalder, J. and Starling, E.H. A note on some factors which determine the blood flow through the coronary circulation. *J. Physiol.* 47: 275-285, 1913.
47. Hilton, R. and F. Eichholtz. The Influence of Chemical Factors on the Coronary Circulation. *J. Physiol.* 59: 413-425, 1925.
48. Anrep, G.V. The Regulation of the Coronary Circulation. *Physiol. Rev.* 6: 596-629, 1926.
49. Elek, S.R. and L.N. Katz. Further Observations on the Action of Drugs on Coronary vessel Caliber. *J. Pharmacol. and Exp. Therap.* 75: 178-182, 1942.
50. Green, H.D. and R. Wegria. Effects of Asphyxia, Anoxia and Myocardial Ischemia on the Coronary blood flow. *Am. J. Physiol.* 135: 271-280, 1942.
51. Eckenhoﬀ, J.E., J.H. Hopkenschiel and C.M. Landmesser. The Coronary Circulation in the Dog. *Am. J. Physiol.* 148: 582-596, 1947.
52. Goodman, L.S. and A. Gilman (1956) The Pharmacological basis of Therapeutics, 2nd Ed. P. 2-8. Macmillan, N. York.
53. Anrep, von G. On Local Vascular Reactions and Their Interpretation. *J. Physiol.* 45: 318-327, 1912.
54. Dale, H.H. and C. Lovatt Evans. Effects on the Circulation of Changes in the Carbon-Dioxide Content of the Blood. *J. Physiol.* 56: 125-145, 1922.
55. Pinkston, J.O., P.F. Partington and A. Rosenblueth. A Further Study of Reflex Changes of Blood Pressure in Completely Sympathectomized Animals. *Am. J. Physiol.* 115: 711-719, 1936.
56. Shires, T. and S.W. Eyer. Studies in Diffusion Respiration. *J. Aviation Med.* 22: 22-30, 1951.
57. Payne, J.P. Hypotensive response to carbon dioxide. *Anaesthesia.* 13: 279-288, 1958.
58. Itami, S. The Action of Carbon Dioxide on the Vascular System. *J. Physiol.* 45: 338-344, 1912.
59. Kaya, R. and Starling, E.H. Note on Asphyxia in the Spinal Animal. *J. Physiol.* 39: 346-353, 1909.
60. Bronk, D.W. and R. Gesell. The Regulation of Respiration. *Am. J. Physiol.* 82: 110-180, 1927.



61. Bernthal, T.G. Some Observations upon changes in Volume Flow of Blood accompanying Changing Respiratory Conditions. Am. J. Physiol. 95: 446-464, 1930.
62. Steck, I.E. and E. Gellhorn. The Effect of Carbon Dioxide Inhalation on The Peripheral Blood Flow in the Normal and In The Sympathectomized Patient. Am. Heart J. 18: 206-212, 1939.
63. Cannon and Hoskins. Am. J. Physiol. 29: 274, 1911 (As cited by Cathcart and Clark. J. Physiol. 49: 300-309, 1914)
64. Cathcart, E.P. and G.H. Clark. The Mode of Action of Carbon Dioxide on the Blood Pressure. J. Physiol. 49: 300-309, 1914.
65. Schaefer, K.E., C.T.G. King, J.L. Mego and E.E. Williams. Effect of a Narcotic Level of CO<sub>2</sub> on Adrenal Cortical Activity and Carbohydrate Metabolism. Am. J. Physiol. 183: 53-62, 1955.
66. Richardson, J.A. and E.F. Woods. Effect of Carbon Dioxide Inhalation on Plasma Concentrations of Epinephrine and Arterenal. Fed. Proc. 15: 473, 1956.
67. Tenney, S.M. Mechanism of Hypertension During Diffusion Respiration. Anesthesiology 17: 768-776, 1956.
68. Müller, J.M., E. Schlittler and H.J. Bein. Reserpin, der sedative Wirkstoff aus Rauwolfia serpentina Benth. Experienta 8: 338, 1952. (Cited by The Rauwolfia Story, Ciba Pharm.)
69. Bein, H.J. The Pharmacology of Rauwolfia. Pharm. Rev. 8: 435-483, 1956.
70. Schneider, J.A. Further studies on the central action of reserpine. Am. J. Physiol. 179: 670-671, 1954.
71. Fletscher, A., P.A. Shore and B.B. Brodie. Release of brain serotonin by reserpine. J. Pharm. and Exp. Therap. 116: 46, 1956.
72. Halzbauer, M. and M. Vogt. Depression by reserpine of the noradrenaline concentration in the hypothalamus of the Cat. J. Neurochem. 1: 8-11, 1956.
73. Muschall, E. and M. Vogt. The action of Reserpine on the peripheral Sympathetic System. J. Physiol. 141: 132-155, 1958.



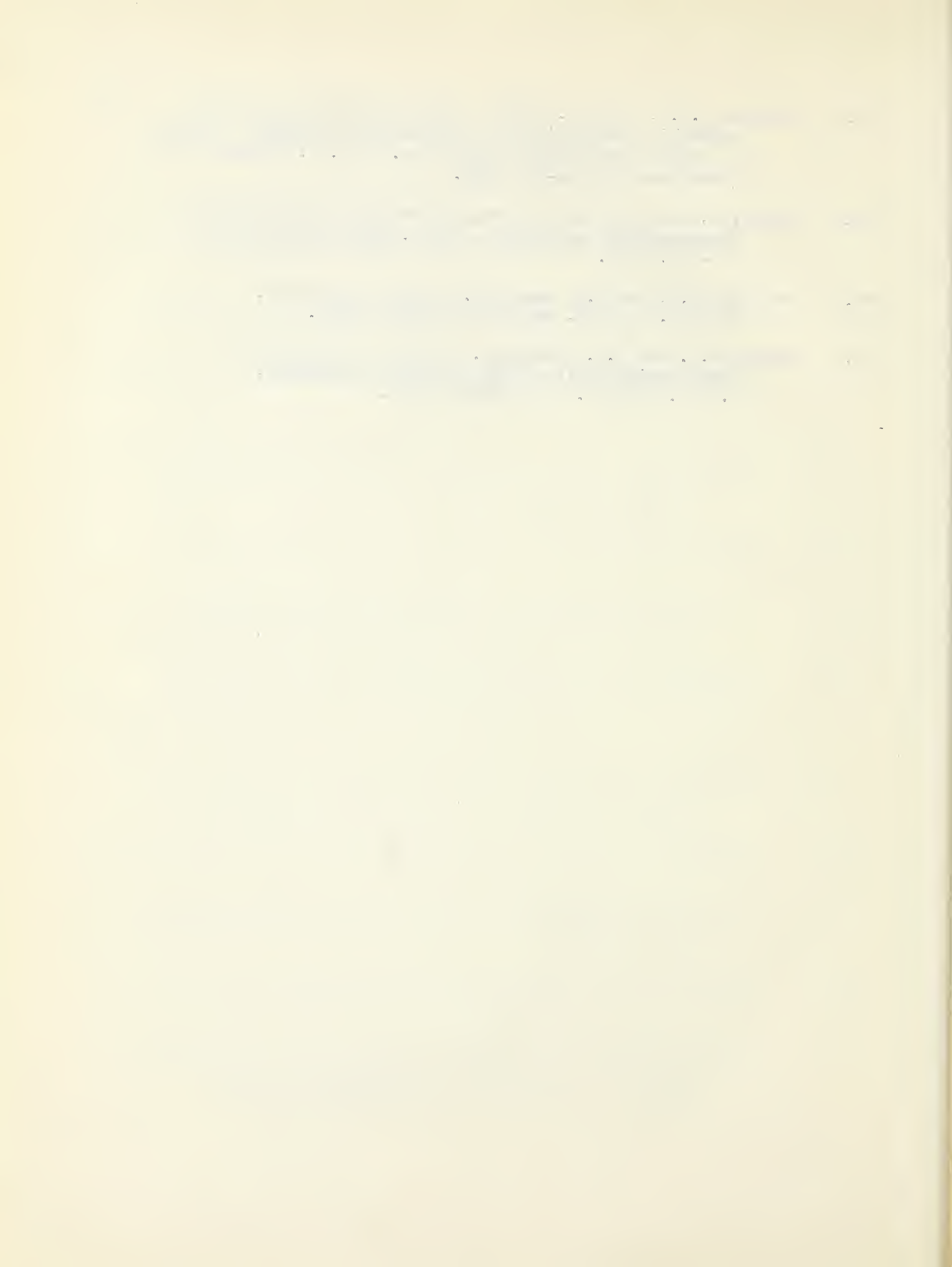
74. McQueen, E.G., A.E. Doyle and F.H. Smirk. The Circulatory Effects of Reserpine. *Circulation* 11, 161-169, 1955.
75. Maxwell, R.A., A.J. Plummer, M.W. Osborne and S. Ross. Evidence for a peripheral action of reserpine. *J. Pharm. & Exp. Therap.* 116: 42, 1956.
76. Maxwell, R.A., S.D. Ross, A.J. Plummer and E.B. Sigg. A peripheral action of Reserpine. *J. Pharm. & Exp. Therap.* 119, 69-77, 1957.
77. Burn, J.H. and M.J. Rand. Noradrenaline in artery walls and its Dispersal by reserpine. *Brit. Med. J.* 1: 903-908, 1958.
78. Euler, V.S. von. Noradrenaline (1956). Charles C. Thomas. Publisher. Springfield, Illinois, U.S.A.
79. Barger, G. and H. H. Dale. Chemical Structure and Sympathomimetic Actions of Amines. *J. Physiol.* 41:19-59, 1910.
80. Swan, H.J.C. Effect of Noradrenaline on the Human circulation. *Lancet.* 257: 508-510, 1949.
81. Collier, H.D., F.H. Meyers and G.H. Schmitt. Hemodynamic Effects of Infusions of Epinephrine and arterenal in in Normal and Shocked animals. *Am. J. Physiol.* 189: 224-228, 1957.
82. Cobbold, A.F. and C.C.N. Vass. Responses of Muscle Blood vessels to Intra-arterially and Intravenously Administered Noradrenaline. *J. Physiol.* 120: 105-114, 1953.
83. Burn, J.H. and D.E. Hutcheon. The Action of Noradrenaline. *Brit. J. Pharm. & Chemotherapy.* 4:373-380, 1949.
84. Dale, H.H. On Some Physiological Actions of Ergot. *J. Physiol.* 34: 163-206, 1906.
85. Ablquist, R.P. A Study of the Adrenotropic Receptors. *Am. J. Physiol.* 153: 586-600, 1948.
86. Furchgott, R.F. The Pharmacology of Vascular Smooth Muscle. *Pharm. Rev.* 7: 183-265, 1955.
87. Burn, J.H. The Action of Tyramine and Ephedrine. *J. Pharm. and Exp. Therap.* 46: 75-95, 1932.
88. Carlsson, A.E. Rosengren, A. Butler and J. Nilsson (1951) Effect of Reserpine on The Metabolism of Catecholamines. Psychotropic drugs pp.368. Ed. Garattini, S. and V. Ghetti. Amsterdam: Elsevier Publishing Company.



89. Oliver, G. and E.A. Schafer. On the Physiological Action of Extracts of Pituitary Body and Certain Other Glandular Organs. *J. Physiol.* 18: 277-279, 1895.
90. Nash, C.W. and J.W. Milligan. An Automic Recording Bubble Flow Meter. Institute of Radio Engineers. Transactions of Medical Electronics. ME-6: 274-276, 1959.
91. Green, H.D., R.N. Lewis and N.D. Nickerson. Quantitation of Changes of Vasomotor Tone. *Proc. Soc. Exp. Biol. @ Med.* 53: 228-229, 1943.
92. Burn, J.H. Practical Pharmacology, p. 35. Blackwell Scientific Publications, 1952. Oxford.
93. Lineweaver, H. and D. Burk. *J. Amer. Chem. Soc.* 56: 658m 1934.
94. Khayutin, V.M. The Recording of Vascular Tone by an Auto-perfusion Method. *Sechenou. Physiol. J. of the U.S.S.R.* 44: 605-613, 1958.
95. Treloar, A.E. Biometric Analysis, an Introduction, 1951, P. 105-107.
96. Chen, G. and D. Russell. A Quantitative Study of Blood Pressure Response to Cardiovascular Drugs and their Antagonists. *J. Pharm. & Exp. Therap.* 99: 401-408, 1950.
97. Kirschmer, L.B. and W.E. Stone. Action of Inhibitors at the Myoneural Junction. *Gen. Physiol.* 34: 821-834, 1951.
98. Martin, G.J. Biological Antagonism, 66. The Blakiston Co. 1951.
99. Brown, G.L., B.N. Davies and J.S. Gillespie. The Release of Chemical Transmitter from The Sympathetic Nerves of the Intestine of the Cat. *J. Physiol.* 143: 41-54, 1958.
100. Furchgott, R.F. The Receptors for Epinephrine and Norepinephrine. *Pharm. Rev.* 11: 429-441, 1959.
101. Belleau, B. Relationships Between Agonists, Antagonists and Receptor Sites. Ciba Foundation Symposium on Adrenergic Mechanisms. 1960.
102. Stephenson, R.P. A Modification of Receptor Theory. *Brit. J. Pharm. & Chemotherapy*, 11: 379-393, 1956.
103. Harvey, S.C. and M. Nickerson. Reactions of Dibenamine and Some Cogeners with Substances of Biological interest in Relation to the Mechanism of Adrenergic Blockade. *J. Pharm. & Exp. Therap.* 112: 274-290, 1954.



104. Graham, J.D.P. and G.P. Lewis. The Antihistamine and Anti-adrenaline Properties of a Series of N- Naphthyl - methyl - 2 - halo - Ethylamine Derivatives. Brit. J. Pharm. & Chemotherapy 8: 54-61, 1953.
105. Paton, W.D.M. A Theory of drug action based on the rate of drug-receptor combination. Proc. Royal Society 154: 21 - 69, 1961.
106. Woolley, D.W. and N.K. Campbell. Serotin receptors. Biochem. Biophys. Acta, 40: 543-544, 1960.
107. Sears, D.F. and R.M. Eisenberg. A Model Representing a Physiological Role of CO<sub>2</sub> at the Cell Membrane. J. Gen. Physiol. 44: 869-887, 1961.



## A P P E N D I C E S

## Abbreviations Used in Appendices

P -- Pressure mm. Hg..

F -- Flow ml per minute.

R -- Resistance (Peripheral resistance units).



## APPENDIX 1

## UNTREATED DOGS

Responses to Noradrenaline (0.05  $\mu$ g/kg, I.A.)

Dog Wt.kg.	P	BEFORE CO <sub>2</sub> Test			% Change			P	DURING CO <sub>2</sub> Test			% Change		
		Basic F	R	P	F	R	R		Basic F	R	P	F	R	R
7.7	80	19.6	4.1	85	7.3	11.6	190	86	17.6	4.8	90	8.7	10.3	110
	83	21.0	3.9	88	8.4	10.4	160	87	17.6	4.9	90	9.2	9.7	90
8.0	115	12.9	8.9	120	3.9	30.7	244	129	24.1	5.4	134	14.0	9.6	77
	113	12.0	9.4	120	3.6	33.3	254	134	24.1	5.6	137	13.4	10.2	82
8.0	127	9.2	13.8	130	5.3	24.5	77	95	5.9	16.1	100	4.5	22.2	38
	117	5.6	20.9	120	4.2	28.5	36	104	5.9	17.6	104	4.2	24.8	41
12.0	137	30.5	4.5	140	14.6	9.6	113	111	6.9	16.1	110	3.9	28.2	75
	128	15.0	8.1	127	5.9	21.5	164	102	11.2	9.1	102	6.7	15.2	67
12.0	122	30.5	4.0	125	12.9	9.6	140	77	10.9	7.0	80	6.7	11.9	70
	86	22	3.8	88	8.4	10.5	176	46	5.8	8.0	50	4.7	10.6	32
11	57	26.9	2.1	59	8.6	6.9	229	49	13.2	3.7	50	6.2	8.1	119
	48	14.9	3.2	49	5.1	9.6	200	52	8.2	6.3	52	4.0	13	106
22	70	19.8	3.6	71	14	5.1	41	65	10.6	6.0	65	7.1	9.1	51
	60	9.2	6.5	60	5.4	11.1	70	56	7.6	7.2	58	4.9	11.8	63
13	176	37.8	4.7	177	16.8	10.5	123	155	19.9	7.8	160	9.8	16.3	108
	70	12.2	7.8	75	5.9	12.7	63	122	13.4	9.2	133	5.6	23.8	159
Mean Values	99	18.7	6.8	102	8.1	15.4	142	92	12.7	8.4	95	7.1	14.7	80



## APPENDIX 1

## UNTREATED DOGS

Responses to Tyramine (10  $\mu$ g/kg, I.A.)

Dog Wt.kg.	BEFORE CO <sub>2</sub>							DURING CO <sub>2</sub>						
	P	Basic F	R	P	Test F	R	% Change R	P	Basic F	R	P	Test F	R	% Change R
12	141	30.8	4.6	142	12.6	11.3	146	105	8.8	11.9	103	3.4	30.3	155
	119	20.1	5.9	117	5.9	19.8	235	98	13.4	7.3	98	5	19.6	168
12	121	24.9	4.8	120	11.2	10.7	123	82	9.0	9.1	86	4.5	19.1	110
	83	24	3.5	87	14	6.2	76	45	4.2	10.7	45	2.0	22.5	110
11	55	21.6	2.6	55	5.6	9.8	277	50	12.2	4.1	50	4.3	11.6	183
	47	11.5	3.9	49	3.7	13.2	238	50	13.4	5.9	51	3.7	13.8	134
22	74	28	2.6	74	16	4.6	76	64	11.4	5.6	65	5.4	12.0	114
	58	9.3	6.2	59	5.8	10.1	62	59	8.8	6.6	60	2.8	21.4	224
13	162	33.6	4.8	162	11.2	14.5	202	158	21	7.5	158	6.2	25.5	240
	66	8.6	7.6	70	2.2	31.8	318	125	16	7.8	134	3.9	34.4	341
Mean Values	93	21.2	4.6	94	8.8	13.2	175	84	11.8	7.6	85	4.1	21	178



## APPENDIX 1

## UNTREATED DOGS

Responses to Pitressin (0.01 unit/kg, I.A.)

Dog Wt.kg.	BEFORE CO <sub>2</sub>							DURING CO <sub>2</sub>						
	P	Basic F	R	Test P	F	R	% Change R	P	Basic F	R	P	Test F	R	% Change R
12	137	22.8	6.0	138	8.4	16.4	173	102	12.7	8.3	103	3.9	26.4	218
	116	13.4	8.6	120	3.5	34.3	299	93	14.6	6.4	93	4.8	19.4	203
12	110	22.4	4.9	113	14.6	8.9	82	86	8.2	10.5	90	8.0	11.2	6
	83	21.0	3.9	85	16.8	5	28	56	5.5	10.2	50	3.8	13.2	29
11	46	7.4	6.2	47	6.0	7.8	26	48	9.9	4.8	48	8.8	5.5	15
	46	14.7	3.1	47	11.2	4.2	35	50	9.7	5.1	50	7.8	6.4	25
22	70	17	4.1	70	15.2	4.6	12	64	10.8	5.8	63	8.4	7.5	29
	59	10.0	5.9	59	7.6	7.7	30	60	10.0	6.0	60	8.1	7.4	23
13	164	20.3	8.0	163	14	11.6	45	160	18.8	8.5	160	16	10	18
	65	9.1	7.2	65	7.3	8.9	24	115	17.6	6.5	120	15.1	7.9	22
Mean Values	90	15.8	5.8	91	10.5	10.9	75	83	11.8	7.2	84	8.5	11.5	59



## APPENDIX 2

## RESERPINE PRETREATED DOGS

Responses to Noradrenaline (0.05  $\mu$ g/kg, I.A.)

Dog Wt.kg.	BEFORE CO <sub>2</sub>							DURING CO <sub>2</sub>						
	Basic			Test			% CHANGE R	Basic			Test			% CHANGE R
	P	F	R	P	F	R		P	F	R	P	F	R	
8	90	23.8	3.8	105	9.2	11.3	197	88	9.2	9.6	90	5.6	16.1	168
	100	11.5	8.7	102	3.6	28.3	225	85	8.4	10.1	87	4.2	20.7	105
10	107	23.6	4.5	112	2.5	44.8	895	61	4.2	14.6	65	2.3	28.3	93
	106	20.0	5.4	107	2.6	41.1	668	75	5.2	14.4	80	3.4	23.5	62
19	73	18.6	3.9	80	2.8	28.5	630	75	13.8	5.4	80	3.7	21.6	296
	92	11.1	8.2	96	2.8	34.3	316	64	6.9	9.2	63	3.3	19.1	106
9.5	53	18.1	2.9	57	3.4	16.7	466	45	8.6	4.5	46	2.6	17.7	240
	45	11.6	3.9	45	3.8	11.8	203	40	7.3	5.4	41	4.8	8.5	56
10	58	14.6	4.0	60	4.8	12.5	212	42	9.0	4.6	43	5.2	8.3	80
13	139	35.4	4.0	147	15.3	9.6	140	120	24.1	5.0	125	5.9	21.2	324
	94	28	3.4	105	3.4	30.9	808	71	15.4	4.6	75	3.6	20.8	352
Mean Values	87	19.7	4.8	92	4.9	24.5	433	70	10.2	7.9	72	4.1	18.7	162



## APPENDIX 2

## RESERPINE PRETREATED DOGS

Responses to Tyramine (10  $\mu$ g/kg, I.A.)

Dog Wt.kg.	BEFORE CO <sub>2</sub>							DURING CO <sub>2</sub>						
	P	Basic F	R	P	Test F	R	% Change R	P	Basic F	R	P	Test F	R	% Change R
10	106	19.9	5.3	113	2.1	53.8	905	66	5.1	12.9	73	2.5	29.2	126
	100	19.9	5.0	107	2.6	41.1	722	75	6.7	11.2	75	3.1	24.2	116
19	62	15.6	4.0	66	1.4	47.1	1070	74	14.6	5.1	79	2.4	32.9	545
	89	19.8	4.5	91	1.3	70	1455	76	17.2	4.4	80	2.6	30.8	592
9.5	53	15.3	3.5	53	2.9	18.3	430	44	8.9	4.9	46	2.8	16.4	231
	47	11.4	4.1	49	2.3	21.3	413	40	6.5	6.1	42	3.4	12.3	100
10	55	16	3.4	56	4.1	13.7	302	46	15	3.1	48	5.6	8.6	177
13	137	26.9	5.1	137	6.7	20.4	300	115	24.1	4.8	123	3.9	31.5	556
	104	20.5	5.1	112	3.4	32.9	545	69	7.8	8.8	75	0.9	83.3	846
Mean Values	84	18.3	4.4	87	3	35.4	682	67	11.7	6.8	71	3	29.9	365



## APPENDIX 2

## RESERPINE PRETREATED DOGS

Responses to Pitressin (0.01 unit/kg, I.A.)

Dog Wt.kg.	BEFORE CO <sub>2</sub>							DURING CO <sub>2</sub>						
	P	Basic F	R	P	Test F	R	% CHANGE R	P	Basic F	R	P	Test F	R	% CHANGE R
8	90	21	4.3	132	4.88	27.7	544	86	9.5	9	100	2.6	38.5	328
10	107	23.2	4.6	130	15.1	8.6	87	70	5.6	12.5	75	2.7	27.8	122
	98	21.0	4.7	109	15.1	7.2	53	73	6.7	10.8	74	3.4	21.7	101
19	80	26.9	2.9	80	4.9	16.3	452	70	14	5.0	71	3.5	20.3	306
	75	21.1	3.5	83	4.8	17.3	387							
9.5	53	14.6	3.6	57	6.0	9.5	164	46	10.1	4.5	46	3.4	13.5	197
	46	19.9	2.3	46	5.9	7.8	247	40	6.9	5.8	41	4.8	8.5	47
10	54	13.7	4.0	54	4.6	11.7	192	44	11.8	3.6	47	8.4	5.6	55
13	116	35.4	3.3	132	11.5	11.5	248	95	22.4	4.2	100	7.6	13.4	214
	105	21	5.0	110	10.9	10.1	102	58	9.1	6.4	67	3.4	19.7	207
Mean Values	82	21.8	3.8	93	8.4	12.8	232	65	10.7	6.9	69	4.4	18.7	175



## APPENDIX 3

Peripheral Vascular Responses to Hypercapnia of Untreated Dogs Before  
and After Tolazoline (2.5 mg/kg, I.V.)

## BEFORE TOLAZOLINE

Dog Wt. kg.	BEFORE CO <sub>2</sub>			DURING CO <sub>2</sub>			% Change R
	P	F	R	P	F	R	
9.5	122	52	2.4	93	11.4	8.2	242
	109	46	2.4	98	12	7.9	229
10	131	58	2.3	121	45	2.7	17
	112	44	2.7	105	31	3.4	26
10.5	135	54	2.5	127	16	7.9	216
	96	13	7.8	115	9.8	12.7	63
10	103	44	2.4	94	28	3.7	54
	107	42	2.5	102	24	4.5	80
Mean Values	114	44	3.1	107	22.1	6.4	116
AFTER TOLAZOLINE (2.5 mg/kg, I.V.)							
9.5	122	24	5.2	86	17	5	-4
	104	18	5.7	102	18	5.7	0
10	104	18	5.8	101	20	5.4	-7
	105	27	4.0	109	24	4.6	15
10.5	114	16	7.3	110	17	6.8	-7
	137	16	8.7	120	12	9.9	14
10	115	50	2.4	82	27	3.1	29
	100	21.7	4.7	97	16	6.0	28
Mean Values	113	23.8	5.5	101	19	5.8	8.5



## APPENDIX 3

Pressor Responses to Noradrenaline (10  $\mu$ g, I.V.) Before and After  
Tolazoline (2.5 mg/kg, I.V.)

Dog Wt.kg.	BEFORE TOLAZOLINE	AFTER TOLAZOLINE
	Response mm. Hg	Response mm. Hg
9.5	20	10
10.0	22	10
10.5	47	25



## APPENDIX 4

Peripheral Vascular Responses to Hypercapnia of Dogs Before and After  
an Acute Dose of Reserpine (1.5 mg/kg, I.V.)

Dog Wt., kg.	BEFORE RESERPINE								
	BEFORE CO <sub>2</sub>				DURING CO <sub>2</sub>				% Change R
	P	F	R	pH	P	F	R	pH	
16.3	143	47	3.1	7.23	123	26	4.7	6.86	52
	122	56	2.2	7.17	114	20	6	6.84	173
15	129	34	3.8	7.29	136	15	9.5	6.94	150
	123	31	3.9	7.25	136	10	14	6.85	246
11	143	51	2.8	7.39	104	22	4.8	6.57	71
	120	42	2.8	7.32	119	26	4.8	6.73	71
13	162	41	4	7.42	178	7	26.7	6.89	567
	147	34	4.3	7.30	160	13	12.7	6.75	195
Mean Values	136	42	3.4		133	17	10.4		190
AFTER RESERPINE (1.5 mg/kg, I.V.)									
16.3	127	44	2.9	7.00	105	20	5.4	6.86	86
	115	32	3.6	7.00	86	14	6.5	6.70	81
15	99	17	5.8	7.15	81	7	11.5	6.72	98
	100	17	5.7	7.10	76	5	14.8	6.70	159
11	116	40	2.9	7.42	104	21	5.1	6.72	76
	110	27	4.2	7.25	106	12	9.1	6.70	117
13	114	28	4.3	7.25	118	10	12.5	6.70	191
	108	19	5.5	7.20	99	10	10.8	6.70	96
Mean Values	111	28	4.4		97	12.4	9.5		113



## APPENDIX 4

Pressor Responses to Pyramine (10  $\mu$ g/kg, I.A.) Before and After  
Reserpine (1.5 mg/kg, I.V.)

Dog Wt.kg.	BEFORE RESERPINE							AFTER RESERPINE						
	Basic			Test			% Change R	Basic			Test			% Change R
	P	F	R	P	F	R		P	F	R	P	F	R	
	96	22	4.4	105	8.4	12.5	184	88	21.5	4.1	105	5	21	412



## APPENDIX 5

Pressor Responses of Untreated Spinal Cats to Intra-Arterial Doses of  
Noradrenaline Before and During CO<sub>2</sub>

Graph	N.A. (Ng)	1/Dose	BEFORE CO <sub>2</sub>		DURING CO <sub>2</sub>	
			Response (mm/Hg)	1/Response	Response (mm/Hg)	1/Response
A	100	0.01	33.8	0.029	18.8	0.053
	200	0.005	46.2	0.021	27.5	0.037
	300	0.0033	53.8	0.018	36.2	0.028
	400	0.0025	73.8	0.013	43.8	0.023
	500	0.002			48.8	0.020
B	62.5	0.016	7.5	0.13	2.5	0.40
	125	0.008	12.5	0.08	5.0	0.20
	250	0.004	12.5	0.08	10.0	0.10
	500	0.002	17.5	0.057	17.5	0.057
	1000	0.001	25.0	0.04	20.0	0.05
C	100	0.01	15.0	0.066		
	500	0.002	32.5	0.030	15.0	0.066
	1000	0.001	100.0	0.01	30.0	0.033
	5000	0.0002	122.5	0.008	85.0	0.012
D	62.5	0.016	8.7	0.11	5.0	0.20
	125	0.008	15.0	0.066	10.0	0.10
	250	0.004	25.0	0.04	12.5	0.08
	500	0.002	30.0	0.033	20.0	0.05
	1000	0.001	45.0	0.022	37.5	0.026
E	100	0.01	25.0	0.04		
	200	0.005	42.4	0.024		
	300	0.0033	52.4	0.019		
	500	0.002	95.0	0.011		
	1000	0.001	157.4	0.0063		



## APPENDIX 6

Pressor Responses of Reserpine Pretreated Spinal Cats to Intra-Arterial  
Doses of Noradrenaline Before and During CO<sub>2</sub>.

Graph	N.A. (Ng)	1/Dose	BEFORE CO <sub>2</sub>		DURING CO <sub>2</sub>	
			Response (mm/Hg)	1/Response	Response (mm/Hg)	1/Response
A	100	0.01	22.5	0.044	45.0	0.022
	125	0.008	23.7	0.042	50.0	0.020
	150	0.006	30.0	0.033		
	200	0.005	35.0	0.028	60.0	0.016
	250	0.004	42.5	0.023	75.0	0.013
	500	0.002	50.0	0.020	100.0	0.010
	1000	0.001	90.0	0.011	140.0	0.007
B	62.5	0.016	17.5	0.057	31.2	0.032
	125	0.008	22.5	0.044	40.0	0.025
	250	0.004	25.0	0.040	50.0	0.020
	500	0.002	30.0	0.033	80.0	0.012
	1000	0.001	35.0	0.028	100.0	0.010
C	62.5	0.016	10.0	0.10	15.0	0.066
	125	0.008	15.0	0.066	17.5	0.057
	250	0.004	22.5	0.044	27.5	0.036
	500	0.002	35.0	0.028	62.5	0.016
	1000	0.001	50.0	0.020	55.0	0.018
D	62.5	0.016	10.0	0.10	17.5	0.057
	125	0.008	15.0	0.066	25.0	0.040
	250	0.004	20.0	0.050	30.0	0.033
	500	0.002	27.5	0.036	32.5	0.030
	1000	0.001	35.0	0.028	40.0	0.025













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